Structure and Function in Diabetic Nephropathy

The Importance of Diffuse Glomerulosclerosis

Derek D. Gellman, M.B., Conrad L. Pirani, M.D., John F. Soothill, B.M., Robert C. Muehrcke, M.D., William Maduros, M.D., and Robert M. Kark, F.R.C.P.
Chicago

Twenty-two years ago, Kimmelstiel and Wilson[1] first described the occurrence of "hyaline masses" in the centers of the glomerular capillary loops in elderly patients with diabetes. They called the lesion "intercapillary glomerulosclerosis." Their illustrations emphasized the circular cross sections of the masses, and thus many later writers added the prefix "nodular" to distinguish these lesions from other varieties of "glomerulosclerosis" seen in diabetes and in other diseases. Kimmelstiel and Wilson drew attention to other clinical features which their patients had in common: hypertension, severe renal failure, proteinuria, and edema which they regarded, on rather scanty evidence, as nephrotic in type.

Once the pathologic lesion had been described other workers began to recognize it and to report its occurrence. It became apparent that many diabetic patients with "intercapillary glomerulosclerosis" did not have the clinical features that Kimmelstiel and Wilson had described,[2-5] and that the majority of them did not have the nephrotic syndrome.[6,7] In part this was because the terms "intercapillary glomerulosclerosis" and "Kimmelstiel-Wilson nephropathy" were broadened to include lesions other than the *nodular* lesions that Kimmelstiel and Wilson had originally described,[7-10] but even those workers who confined their attention to the nodular lesions[3-5] were unable to delineate with certainty an accompanying clinical syndrome. On the other hand, it became equally apparent that the nephrotic syndrome could occur in diabetic patients in whom nodular glomerulosclerosis was absent or very mild.[11-14]

In 1942, Fahr[15] and others[9,16] drew attention to the frequent occurrence of another glomerular lesion in diabetics — diffuse glomeruloscerosis. It has been suggested[17] that this diffuse lesion, not the nodular lesion of Kimmelstiel and Wilson, is the cause of massive proteinuria and the nephrotic syndrome in diabetes. In this paper, we shall briefiy discuss some of the relationships between the clinical features and the nodular and diffuse lesions found in a group of patients studied by percutaneous needle biopsy of the kidney. A more complete description of our findings in diabetic nephropathy will be published elsewhere.[18]

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From the Departments of Medicine and Pathology, University of Illinois College of Medicine, and Presbyterian-St. Luke's Hospital, Cook County Hospital and the Research and Educational Hospital, Chicago, Illinois.

MATERIALS AND METHODS

Fifty-three patients* with diabetes were studied—twenty-eight males and twenty-five females. They were not an unselected sample of the total diabetic population: To obtain such a sample would be impossible. The patients were selected because they had clinical evidence of renal involvement, or because they were cooperative patients in whom we hoped to be able to study the onset and progression of renal involvement. The proportion of patients with clinical evidence of diabetic nephropathy was high, and no patients with "juvenile" diabetes of short duration were studied (figure 1). However, we believe that selection of this sort does not invalidate our conclusions regarding the relationship between the severity of the histological lesions and the results of biochemical measurements and tests of renal function.

Sixty-three biopsies were made. Shortly before each biopsy the clinical state of the patient was recorded, and a number of simple tests of renal function were carried out. The renal tissue was fixed and stained by the conventional technics described previously:[19] Serial sections were examined in almost every case. The severity of

*In this group of patients, renograms were not done to determine whether or not the hypertension might have been due to ischemia associated with arteriosclerotic lesions of the renal arteries or their major branches. It is unlikely that any of them suffered with such lesions, as other satisfactory causes were found in the biopsies for their hypertension.

nodular glomerulosclerosis and of diffuse glomerulosclerosis was graded independently on a range of 0- to 4-plus units by two or three of us (D.D.G., C.L.P., and J.F.S.). The criteria used in allotting grades are outlined in the Addendum and will be given in greater detail elsewhere.[18]

HISTOLOGY

Nodular diabetic glomerulosclerosis (figure 2). The morphology of these lesions has been well described by Kimmelstiel and Wilson,[1] Allen[20] and Reid.[21] They consist of laminated hyaline masses, usually situated at the periphery of the glomeruli. They are surrounded either by a patent capillary or, in more advanced stages, by layers of nuclei arranged in an "onion-skin" formation. It is unusual to find nuclei in the center of the nodule, but two nodules may coalesce to give that appearance.

Diffuse diabetic glomerulosclerosis (figure 3). Few authors other than Bell[14,16] have paid much attention to this lesion. Briefly, the lesion consists of thickening of the capillary walls by eosinophilic material which also takes the Periodic Acid Schiff stain. All aspects of the capillary wall are involved so that in cross section the capillaries appear as thickened rings, and the lumina are widely separated one from the other by the abnormal material. As the lesion becomes more severe the capillary lumina become progressively narrower, until the whole glomerulus is ischemic. The glomeruli remain large, in contradistinction to the ischemic glomeruli of arteriolar nephrosclerosis. The distinction from membranous glomerulonephritis can be made by attention to three points. First, diffuse diabetic glomerulosclerosis is far less uniform a lesion than is membranous glomerulonephritis. Second, the abnormal material in the basement membrane soon spreads to surround the endothelial cells: This does not happen in membranous glomerulonephritis. Third, hyalination of the afferent (and efferent) arterioles is a prominent feature of diffuse diabetic glomerulosclerosis, but is not seen in membranous glomerulonephritis unless severe hypertension is also present.[14,18]

STRUCTURAL AND FUNCTIONAL RELATIONSHIPS

Nodular glomerulosclerosis was never found in the absence of the diffuse lesion, but diffuse lesions were seen in eighteen biopsies in which nodules could not be found despite careful search. In those specimens containing both types of lesion there was no correlation between their severity. In one patient with the nephrotic syndrome no nodules could be found in two biopsies (figure 4).

The mean level of the diastolic blood pressure was higher in patients with nodular glomerulosclerosis (mean: 101.8; standard error: 3.2) than in those without nodular glomerulosclerosis (mean 81.1; standard error: 2.0). However, there was no correlation between the *severity* of the nodular lesion and the blood pressure. On the other hand, there was a progressive, and statistically significant, increase of diastolic blood pressure with increasing severity of the diffuse lesion (figure 5, table 1). This relationship was apparent in all age groups.

The serum levels of urea nitrogen and creatinine and the amount of proteinuria progressively increased with

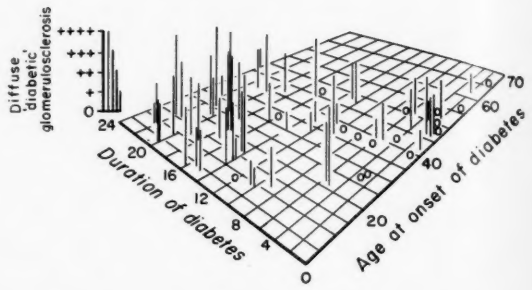

FIG. 1. The severity of diffuse glomerulosclerosis in sixty-three renal biopsies and nine autopsies in a group of fifty-three patients, plotted against age of onset and duration of diabetes. No patients with onset before twenty and duration less than eight years were studied.

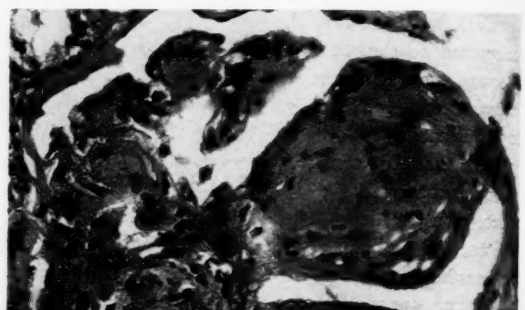

FIG. 2. Nodular diabetic glomerulosclerosis.
Note the lamination of the nodules, and the remains of a capillary around each one. Typically the nuclei are confined to the periphery of the nodules, but sometimes two or more nodules coalesce (as in this example) so that the nuclei appear to be centrally placed (hematoxylin and eosin, reduced from 500 magnification).

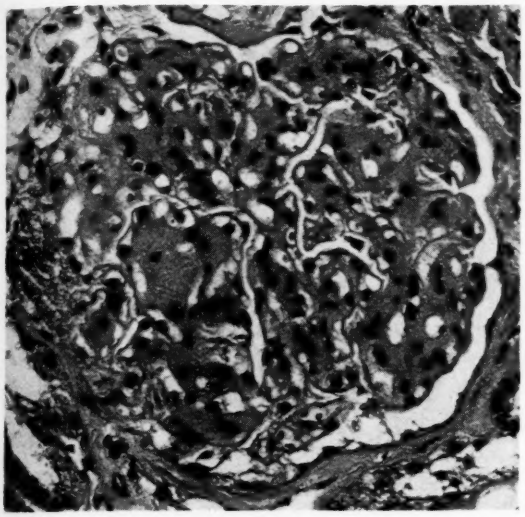

FIG. 3. Diffuse diabetic glomerulosclerosis.
The glomerulus is large and appears distended with "hyaline" material in which the nuclei are uniformly distributed. Many of the capillaries appear as rings with thickened walls (hematoxylin and eosin, reduced from 500 magnification).

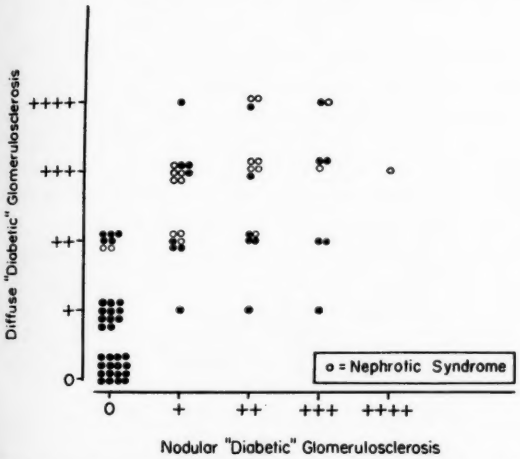

FIG. 4. Each circle represents the severity of nodular and diffuse glomerulosclerosis in one of sixty-three renal biopsies and nine autopsies in a group of fifty-three patients with diabetes. The patients designated by open circles had the nephrotic syndrome in addition to diabetes (see text).

increasing severity of diffuse glomerulosclerosis, but were not related to the severity of nodular glomerulosclerosis (table 1).

The serum albumin levels and the creatinine and urea clearances (figure 6) progressively decreased as the diffuse lesions became more severe but were not correlated with the severity of nodular glomerulosclerosis (table 1).

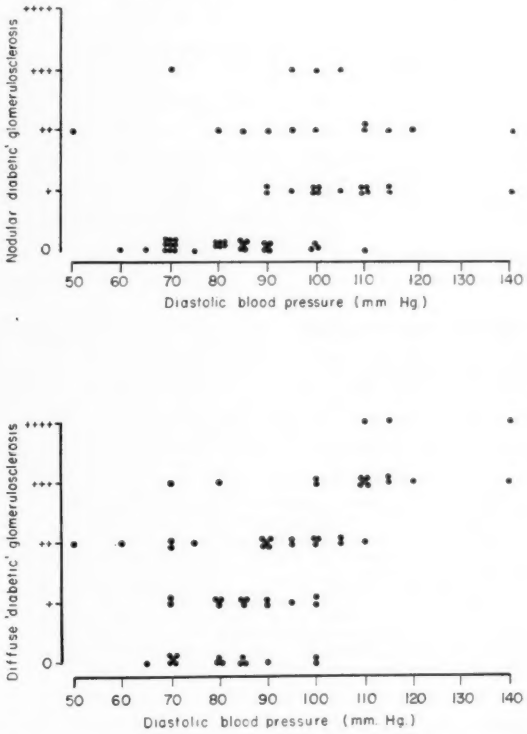

FIG. 5. The relationship between diastolic blood pressure and nodular and diffuse diabetic glomerulosclerosis (see text and table 1).

A progressive increase in the proportion of patients with edema and the nephrotic syndrome occurred as diffuse glomerulosclerosis became more severe. There was no corresponding increase as the nodular lesion became more severe (figure 7).

DISCUSSION

Nodular glomerulosclerosis is, in its early stages, a "focal" lesion: That is to say, it affects only a small proportion of glomeruli. A study of autopsy specimens shows that the affected glomeruli are distributed in a random fashion throughout the kidney, and not grouped together as are, for example, the affected glomeruli in focal pyelonephritis. It is certainly possible that in sampling groups of between five and twenty glomeruli (the number most frequently obtained by the needle biopsy technic) some of the early cases were missed, but presumably in such missed cases not more than one glomerulus in five, on the average, was affected by

TABLE 1

Correlations between the presence and severity of nodular and diffuse diabetic glomerulosclerosis and clinical findings and laboratory tests*

Test	No. of cases	Nodular glomerulosclerosis: Direction of correlation	Nodular glomerulosclerosis: Probability	Diffuse glomerulosclerosis: Direction of correlation	Diffuse glomerulosclerosis: Probability
1. Diastolic blood pressure	61	Positive	<0.001	Positive	<0.001
		Negative†	>0.3	Positive†	<0.001
2. Proteinuria	40	Positive	>0.2	Positive	<0.01
3. Serum albumin	61	Negative	<0.01	Negative	0.00001
		Positive†	>0.5	Negative†	<0.01
4. Serum urea nitrogen	54	Positive	<0.001	Positive	0.00001
		Positive†	>0.3	Positive†	<0.001
5. Serum creatinine	49	Positive	<0.01	Positive	<0.001
		Negative†	>0.8	Positive†	<0.01
6. Urea clearance	50	Negative	>0.05	Negative	<0.001
7. Creatinine clearance	35	Negative	<0.02	Negative	<0.02
		Negative†	>0.5	Negative†	<0.02

*Data such as those shown in figures 5 and 6 do not readily lend themselves to the calculation of correlation coefficients by conventional methods, because they deal with the association of a numerical characteristic with a ranked characteristic. The method we have used is, in essence, a comparison of the direction of the slopes between an infinite number of pairs of the points, chosen at random, adjusted for the appropriate number of degrees of freedom. The method indicates whether there is a positive or negative correlation, and if so, the probability that it might have occurred by chance.[22] We are indebted to Dr. William R. Best for his help in the handling and analysis of the data.

†The results marked with this symbol were obtained by excluding those patients in whom the histological lesion was absent. They therefore give a more accurate representation of the relation between *increasing severity* of the histological lesion (as distinct from its mere presence or absence) and the test under consideration.

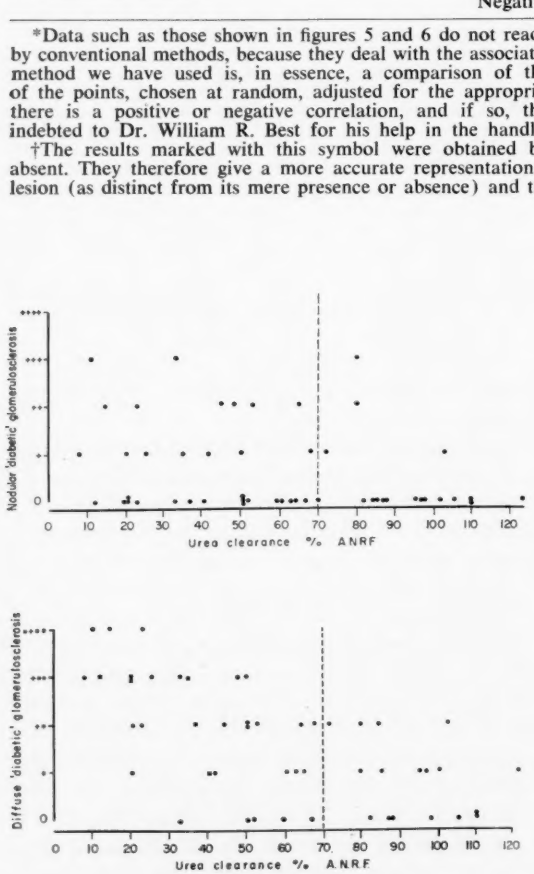

FIG. 6. The relationship between urea clearance, expressed as a percentage of "Average Normal Renal Function," and nodular and diffuse diabetic glomerulosclerosis (see text and table 1).

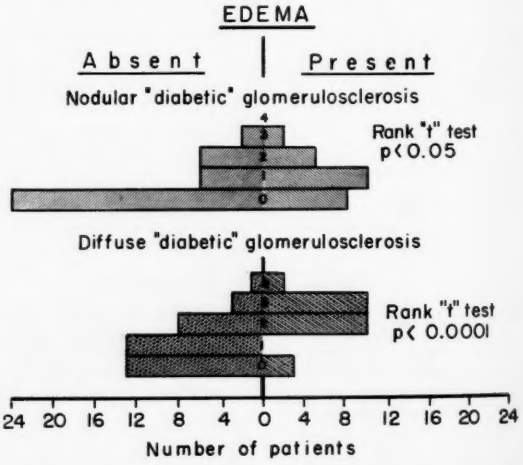

FIG. 7. The relationship between edema and nodular and diffuse diabetic glomerulosclerosis. Each block shows the number of patients with and without edema with the grade of glomerulosclerosis indicated therein. The apparently significant relationship with nodular glomerulosclerosis ($0.05 > p > 0.045$) depends entirely upon the large group without nodules (see text).

nodules. In view of what is known of the functional reserve of the kidney, it is difficult to understand how such focal lesions could impair renal function.

It is reasonable to suppose that if a pathologic lesion is the cause of a functional abnormality in an organ, then, as the lesion becomes more severe the function abnormality will become progressively worse. The data presented in this paper show that the components of what is frequently called "The Kimmelstiel-Wilson Syndrome" —namely, hypertension, renal failure, and proteinuria and its consequences (the nephrotic syndrome), in patients with diabetes—are *not* associated in this way with the severity of the accompanying nodular diabetic glomerulosclerosis. They are related in a progressive manner to the severity of diffuse diabetic glomerulosclerosis.

SUMMARY AND CONCLUSIONS

Sixty-three renal biopsies taken from fifty-three diabetic patients with and without clinical evidence of renal involvement were studied. The severity of nodular diabetic glomerulosclerosis and of diffuse diabetic glomerulosclerosis in each was assessed, and correlated with the clinical features and the results of renal function tests.

Diffuse glomerulosclerosis was found in the absence of the nodular lesion, but nodular glomerulosclerosis was never found in the absence of the diffuse lesion. Hypertension, renal failure, and proteinuria and its consequences (the nephrotic syndrome) were quantitatively correlated with the *severity* of diffuse glomerulosclerosis, but not with the *severity* of nodular glomerulosclerosis.

Much confusion would be avoided if the eponym "Kimmelstiel-Wilson" were confined to a histologic lesion (*nodular* diabetic glomerulosclerosis) and not applied to a clinical syndrome, because the one does not appear to be the cause of the other.

SUMMARIO IN INTERLINGUA

Structura E Function In Nephropathia Diabetic: Le Importantia De Glomerulosclerosis Diffuse

Esseva studiate sexanta-tres biopsias renal ab cinquanta-tres patientes diabetic con o sin manifestationes de affection renal. In omne caso individual, le severitate del glomerulosclerosis diabetic nodular esseva evalutate e correlationate con le aspectos clinic e le resultatos de tests de function renal.

Glomerulosclerosis diffuse esseva trovate in le absentia del lesion nodular, sed glomerulosclerosis nodular nunquam esseva trovate in le absentia del lesion diffuse. Hypertension, disfallimento renal, e proteinuria e su consequentias (i.e. le syndrome nephrotic) se monstrava correlationate quantitativemente con le severitate de glomerulosclerosis diffuse sed non con le severitate de glomerulosclerosis nodular.

Multe confusion poterea esser evitate si le eponymo "Kimmelstiel-Wilson" esseva restringite a un lesion histologic (i.e. a glomerulosclerosis diabetic nodular) e non applicate a un syndrome clinic, proque il pare que le un non es le causa del altere.

ACKNOWLEDGMENT

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Figures 2 and 3 are published by the kind permission of the editors of the *Annals of Internal Medicine.*

ADDENDUM

Grading of Nodular Diabetic Glomerulosclerosis

One plus: single nodule in occasional glomeruli.

Two plus: nodules in many glomeruli, but many spared.

Three plus: nodules in most glomeruli, but only a few completely ischemic.

Four plus: most glomeruli made ischemic by nodules.

Grading of Diffuse Diabetic Glomerulosclerosis

One plus: "local thickening of capillary walls; few glomeruli involved.

Two plus: diffuse thickening of capillary walls; nearly all glomeruli involved.

Three plus: local ischemia of glomeruli due to obliteration of capillary lumina.

Four plus: widespread ischemia of glomeruli.

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Since the introduction of practical methods for the production and measurement of substances containing isotopes of elements common in biological systems—such as hydrogen, carbon, nitrogen, and phosphorus—these have found expanding uses as "tracers" in biological systems. For the identification of intermediate compounds and the qualitative description of biochemical pathways and reaction mechanisms, they have allowed unprecedented advances; and some isotopically labeled substances also have been useful in analytical measurements—by the "isotope dilution" principle—of the quantities of material present or of their volumes of distribution in biological systems. A further type of use, of great potential importance theoretically, has often been attempted: by observation of the "movement" of labeled material to measure the absolute or relative *rate* of movement or of chemical transformation of unlabeled substance already present in the system. To the uninitiated in this field, this process would seem to be quite simple in principle. A substance containing a known quantity of the isotopic element would be introduced into the animal or surviving metabolizing system, and after a time one would find out how much of the isotope was present in a particular place or form. This might be feasible if one were dealing only with a single simple transfer of material from one "compartment" of known dimensions to another. In biological systems, however, this is very seldom the case. Instead, one finds that the transfer may be reversible; that what appears to be a simple transfer is in fact a series of reactions (some of them reversible, perhaps); that the substance introduced is itself being replaced with nonisotope material as fast as it is used; or that it can exchange with not one but many other forms. Hence the interpretation of rates of movement of the isotope in terms of the rates of movement of unlabeled materials is seldom simple and may be deceptively complicated.

Jane A. Russell, in "The Use of Isotopic Tracers in Estimating Rates of Metabolic Reactions," from *Perspectives in Biology and Medicine* 1:138, Winter, 1958.

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Biochemical Changes in Skeletal Muscle in Patients with Uncontrolled Diabetes Mellitus

J. A. Litchfield, M.D., Walsall, England

The disturbances of water and electrolyte metabolism which may occur in patients with uncontrolled diabetes mellitus have received extensive investigation[1] by balance studies, and the occurrence of cellular dehydration[2,3] and potassium loss[4] in diabetic patients with ketosis suspected from such observations have been confirmed.[5,6]

With the object of seeking direct verification of the chemical changes occurring in tissues in such cases, Knowles and Guest[7] analyzed muscle samples removed from alloxan-diabetic rats which had been allowed to develop ketoacidosis. Their analytical data showed a reduction in the muscle contents of water and potassium. Values derived from the analytical data showed cellular dehydration and no change in the concentration of potassium in cell water. The present work was undertaken to determine whether similar changes occurred in the composition of muscle in humans with uncontrolled diabetes.

MATERIALS AND METHODS

Four adult male patients admitted consecutively to hospital for stabilization of their diabetes were selected for the study. None had vomited, none had previously received insulin and all had polyuria and polydipsia to variable degrees. The patient with the most severe degree of ketosis, (J.G.), was slightly drowsy, but there was no disturbance of consciousness in the other three. Brief summaries giving an indication of the clinical status of the patients are included in the appendix.

On the first day of admission to hospital and before the commencement of treatment, a sample of quadriceps femoris muscle was taken under local anesthesia and analyzed for water, neutral fat, nitrogen, sodium and potassium by the method of Litchfield and Gaddie.[8] Directly after the operation a sample of blood was withdrawn with minimal stasis from an antecubital vein and allowed to clot. The serum was separated and analyzed for water by drying to constant weight; for sodium and potassium using the Eel flame photometer; and chloride by the method of Schales and Schales.[9] The CO_2 combining power was determined on serum from blood drawn into an oxalated tube under paraffin by the method of Peters and Van Slyke.[10]

Blood glucose was determined by the method of Hagedorn and Jensen.[11]

Calculations: The results of the serum analyses have been calculated and expressed in terms of serum water since the serum of diabetic patients contains excessive amounts of fat.[12] The osmolar concentrations of serum sodium and the glucose present in excess of 100 mg./100 ml. serum have been calculated by the method described by Welt[13] and expressed also in terms of serum water.

The constituents of total muscle have been expressed per unit weight of dry fat free solids. Extracellular water was calculated assuming the distribution of chloride to be restricted to the extracellular phase and its concentration in that phase to be that existing in an ultrafiltrate of plasma. A Gibbs-Donnan factor of 0.95 was used. A further correction factor of 0.99 was applied when converting phase values from volumetric to gravimetric units since the water of the extracellular phase contains about 1 per cent of solids. The water content of the intracellular phase was estimated by subtracting extracellular water from total muscle water, and after correcting by subtraction for the amounts of sodium and potassium outside the cells, the intracellular concentrations of these ions were derived.

RESULTS

I. *Serum Analyses*: In the patients E.B., D.J., and R.P., the concentrations of serum water sodium were low, or low normal (table 1); in J.G., however, the sodium concentration was elevated to 168 mEq./L. serum water. The osmolar concentration of sodium + glucose was raised in this patient to 184 m.Osm. and within normal limits in the other three patients.

II. *Muscle Analyses.*

(a) *Water.* The total muscle water contents were reduced in all the samples except that of E.B. (table 2).

Consulting Physician, Walsall Group of Hospitals, England. (Formerly Senior Medical Registrar, The General Hospital, Birmingham, England.) Present address: Manor Hospital, Walsall, Staffordshire, England.

TABLE 1

Serum values

Subject	H_2O gm./L. serum	Glucose gm./100 ml. serum water	Per liter of serum water: Na mEq.	Cl mEq.	K mEq.	CO_2 mEq.	Glucose mM.	Osm. conc. mOsm.
R.P.	843	540	144	105	5.9	—	30	159
J.G.	888	574	168	123	5.8	23.2	32	184
E.B.	939	353	136	113	5.1	27.6	19	143
D.J.	929	307	142	108	5.1	26.9	17	150

The normal range for serum water osmolarity in terms of sodium is 148-160 mOsm.

Intracellular water was reduced in R.P., J.G., and E.B. to 695, 690 and 702 gm./kg. cells respectively (table 3). In D.J., intracellular water was 719 gm./kg. cells (normal 731, S.D. 10.5) and was within normal limits.

(b) *Potassium.* In the patient J.G., muscle potassium was reduced to 35.2 mEq./100 gm. fat-free solids; in the other three patients muscle potassium contents were within the normal range (table 2). The derived data (table 3) show that the intracellular concentrations of potassium were all within the normal range.

(c) *Nitrogen.* The amounts of noncollagen nitrogen (NCN) associated with 100 gm. fat-free solids were normal in the three samples in which this determination was made and the K/NCN ratios were within normal limits (table 2).

(d) *Sodium.* The amounts of sodium associated with 100 gm. fat-free solids were normal in J.G., E.B,. and D.J. (table 2) and probably high normal in R.P. (18.1 mEq./100 gm. fat-free solids).

TABLE 2

Muscle analyses: Analytical data per 100 gm. fat-free tissue solids

Subject	H_2O gm.	Cl mEq.	Na mEq.	K mEq.	NCN gm.	K/NCN
R.P.	310	9.2	18.1	40.3	12.8	3.15
J.G.	282	5.3	17.6	35.2	11.9	2.96
E.B.	329	10.0	15.7	42.7	—	—
D.J.	310	6.3	12.5	43.1	12.9	3.34
Normal* Mean	339	8.1	13.9	44.7	13.4	3.40
S.D.	11.0	1.4	1.9	2.5	1.1	0.29

NCN = noncollagen nitrogen.
*Normal values calculated from muscle analyses performed on twenty-three normal subjects.

DISCUSSION

The results of the present series of muscle analyses are in accordance with those of Knowles and Guest[7] in demonstrating depletion of intracellular water in the presence of hyperglycemia. The inspection of the present data suggests, furthermore, a possible correlation between the heights of the blood sugar levels and the degrees of cellular dehydration, but unfortunately the series is too small to permit statistical evaluation. On the other hand, the serum osmolarity was elevated only

TABLE 3

Muscle analyses: derived data

Subject	Gm./kg. fresh fat-free muscle: extracellular water	intracellular water	Gm./kg. cells: intracellular water	mEq./kg. intracellular water: K	Na	(K+Na)
R.P.	207	550	695	176.3	24.7	201.0
J.G.	119	607	690	158.2	46.5	204.7
E.B.	222	545	702	180.5	8.8	189.3
D.J.	139	618	719	169.4	16.8	186.2
Normal Mean	157	615	731	164.3	10.8	175.1
S.D.	25.5	25.8	10.5	6.3	6.5	7.1

in J.G. who was the most distressed by thirst and in whom intracellular dehydration was greatest; in the other three patients serum sodium levels were low, resulting in apparently normal serum osmolarity despite the presence of hyperglycemia.

Explanations for the foregoing observations may be found in the works of Seldin and Tarail[14] and Tarail and associates[15] who showed that by raising the osmotic pressure of the extracellular fluid with the rapid infusion of hypertonic glucose solution, serum sodium concentration was depressed to an extent greater than could be accounted for by the administered solution, or by the renal excretion of sodium. It was concluded that since glucose does not readily enter cells[16] and that any which does is quickly metabolized, an osmotic gradient is set up across the cell membrane resulting in the transfer of water to the extracellular phase with consequent intracellular dehydration. The present work suggests that this new state of equilibrium may continue to exist with the persistence of hyperglycemia.

Conclusive evidence of deficiency of total muscle potassium was found only in J.G., and there was no reduction in the intracellular concentrations of potassium in any of the patients. Conversely in this patient who was the most thirsty and whose blood sugar level was the highest of the series, intracellular sodium was elevated to 46.5 mEq./kg. intracellular water. Although sodium is known to move into cells in states of potassium deficiency[17,18] this does not appear to have been the mechanism in the patients under present discussion. It seems more probable that hypertonicity of the extracellular fluid due to hyperglycemia induced this transfer as suggested by Elkinton and associates.[19] On the other hand, the extent to which the breakdown of cellular metabolism due to deficiency of insulin[20] played a part cannot at present be evaluated.

SUMMARY

Muscle analyses were performed on four male patients with uncontrolled diabetes mellitus.

The results showed reductions of intracellular water to 695, 690 and 702 gm./kg. cells in the patients with blood sugar levels of 540, 574 and 353 gm. glucose/100 ml. serum water respectively. In the patient D.J. who had the lowest blood sugar level of 307 gm./100 ml. serum water cellular hydration was within normal limits. Reasons are given for attributing these findings to hyperosmolarity of the extracellular phase due to the presence of abnormally large amounts of glucose.

There was no change in the intracellular concentrations of potassium although in the most severely affected patient the whole muscle content of potassium was reduced to 35.2 mEq./100 gm. fat free tissue solids. No definite explanation for the increased amount of intracellular sodium found in this patient can be given, but the results of the above work are generally concordant with those of other authors using balance technics.

SUMMARIO IN INTERLINGUA

Alterationes Biochimic in Musculo Skeletic de Patientes con Nonstabilisate Diabete Mellite

Analyses de musculo esseva effectuate in quatro masculos con nonstabilisate diabete mellite.

Le resultato del analyses monstrava reductiones del aqua intracellular usque al nivellos de 695, 690, e 702 g per kg de cellulas in le tres patientes con nivellos de sucro de sanguine de 540, 574, e 353 g de glucosa per 100 ml de aqua seral, respectivemente. In le patiente D.J., qui habeva le plus basse nivello de sucro de sanguine (307 g per 100 ml), le hydratation cellular con aqua seral esseva intra le limites del norma. Es presentate rationes que justifica attribuer iste constatationes al hyperosmolaritate causate in le phase extracellular per le presentia de anormalmente grande quantitates de glucosa.

Esseva notate nulle alteration del concentration intracellular de kalium, ben que in le plus severmente afficite patiente, le contento de kalium in le musculo total esseva reducite a 35,2 mEq per 100 g de non grassose solido histic. Nulle explication definite pote esser formulate pro le augmento del natrium intracellular in le caso de iste patiente, sed le resultatos del presente investigationes se trova de accordo general con le resultatos obtenite per altere autores per medio de technicas de balancia.

ACKNOWLEDGMENT

I wish to thank Dr. R. Gaddie of the General Hospital, Birmingham, England, for his assistance with the biochemical technics, and Dr. J. M. Malins of the General Hospital, Birmingham, England, for allowing me access to his patients.

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APPENDIX

Clinical Summaries of the Four Patients Studied

R.P., aged nineteen, male. For two months before admission he complained of increasing thirst and polyuria. During the same period he had frequent nocturnal cramps in the legs. He had lost about forty pounds in weight. On admission on Oct. 26, 1955, a moderate degree of dehydration was shown by dryness of the mouth and diminished tissue turgor; the blood pressure was 130/80 mm. Hg. The urine contained about 2 per cent of sugar and a moderate amount of acetone.* Control of his diabetes proceeded without difficulty and intravenous fluids were not required.

J.G., aged twenty, male. For two months this man complained of excessive thirst and polyuria before being seen in consultation; he had lost about fifty pounds in weight. On admission on Nov. 30, 1955, he was markedly dehydrated, and early peripheral circulatory failure was present. The blood pressure was 112/70 mm. Hg. The patient was drowsy and air hunger was present. The urine contained over 2 per cent of sugar and the urine test for acetone was strongly positive. He was given two liters of normal saline intravenously during the first three hours, following which he was stabilized by conventional methods.

E.B., aged forty-six, colored male. The patient complained of thirst and polyuria of two months' duration; he had lost weight, but to what extent was unknown. On admission on Dec. 13, 1955, slight dryness of the mouth was present, but other clinical evidence of dehydration was absent; the blood pressure was 120/75. The urine contained 2 per cent of sugar and no acetone. No difficulty was experienced during control.

D.J., aged twenty-eight, male. The history of progressive thirst and polyuria was of six weeks' duration and the patient had lost about forty pounds in weight. He was admitted to hospital on Jan. 2, 1955, at which time there was no clinical evidence of dehydration; the blood pressure was 120/80 mm. Hg. Respiration was normal. The urine contained 2 per cent of sugar and the urine test for acetone was positive. Intravenous fluid therapy was unnecessary and control was carried out without complications.

Although an accurate comparison of the severity of the metabolic disturbances in these four patients cannot be made on clinical grounds alone, it is possible to say that J.G. was the most dehydrated and that in D.J. clinical signs of dehydration were lacking. R.P. and E.B. were slightly dehydrated and, of the four patients studied, E.B. was the only one in whom ketosis was absent. Thirst appears to have been the most severe in R.P. and J.G.

*Urine sugar was estimated by the Clinitest (Ames) reagent and acetone by the Acetest (Ames) reagent.

Nonatheromatous Peripheral Vascular Disease of the Lower Extremity in Diabetes Mellitus

Sidney Goldenberg, M.D., Morris Alex, M.D., Ram A. Joshi, M.B., and Herman T. Blumenthal, Ph.D., M.D., St. Louis

Essential to any effective approach to the prevention and therapy of the vascular disease associated with diabetes is an understanding of its etiology and pathogenesis. In this regard two possibilities have generally been considered: (1) The vascular complications of diabetes, with the possible exception of the lesions in retinal vessels and renal glomeruli, are due to intensification of ordinary arteriosclerosis; and (2) they are due to a disseminated vascular disease more directly related to the diabetes, of which the retinal vascular and renal glomerular lesions are representative. It would appear that before any real light can be shed on this problem, it is first necessary to set up criteria by which one may distinguish lesions which represent an arteriosclerotic process from those which are of a different nature.

In a previous study[1] it was shown that arteriosclerosis of the lower extremity is characterized by a diminishing intensity of the vascular lesions as the arteries diminish in size. Thus the iliac and femoral arteries show the most advanced stages, the popliteal and tibial arteries less advanced disease, the dorsalis pedis only minimal sclerosis, and the digital arteries only the very beginnings of the arteriosclerotic process. On a microscopic level sclerotic lesions are first characterized by a splitting, fraying and reduplication of the internal elastic lamella and the laying down of mucopolysaccharides as the intima develops. Later, lipids accumulate, until with advanced plaque formation they become a prominent component of the lesion. In the media calcification and, later, bone formation occur (Mönckeberg sclerosis), and these processes also diminish in intensity with decreased size of arteries, until there is no significant calcification in the digital vessels. Arteries smaller than the digitals usually show no lesions except in hypertensive subjects, where they are characterized by fibrosis with hyalinization of the intima and hypertrophy and hyalinization of the media. At no level is endothelial proliferation a characteristic of the arteriosclerotic process. It is these processes which most investigators believe are intensified in diabetes and account for the increased incidence of gangrene in diabetic patients.

In setting up criteria for the recognition of a generalized diabetic vascular disease we have drawn upon the experience of other investigators who have studied the renal glomerulus and retinal arteries. Two basic components of such vascular lesions have been described: (1) McManus,[2] Koss,[3] Friedenwald[4] and others have emphasized the appearance of a Periodic Acid-Schiff positive substance in the renal and retinal lesions, and (2) Ballantyne and Lowenstein[5] have described an initial swelling and proliferation of the endothelium of the retinal vessels. More recently, studies by Bergstrand and Bucht,[6] Irvine et al.,[7] and by Rinehart et al.[8,9] with the electron microscope, have revealed both a thickening and a folding of the capillary basement membrane of glomeruli in diabetic glomerulosclerosis accompanied by a precipitation of a "hyaline" material derived from the cytoplasm of the endothelium, and a proliferation of the latter cells.

In discussing the nature of the retinal and glomerular vascular lesions believed to be directly related to diabetic disease, Friedenwald[4] remarked that it seemed indeed strange that such lesions are limited only to the eyes and kidneys, and he believed that "other organs have as yet not been adequately explored to disclose the full extent of this vascular disease." Recently, Burstein, Blumenthal and Soule[10] have found vascular lesions in the placentas of diabetic mothers which have characteristics analagous to those found in the retinal and renal glomerular vessels.

It is primarily the purpose of this report to present the pathologic characteristics of similar vascular lesions of the lower extremity, which also appear to have the same degree of relatedness to diabetes as those found in the

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From the Institute of Experimental Pathology and the Division of Medicine, The Jewish Hospital, The Departments of Pathology and Medicine, St. Louis University School of Medicine, and the Department of Medicine, Washington University, School of Medicine, St. Louis, Missouri.

retina and glomerulus. The vessels in 152 amputation specimens of various portions of the lower extremity have been studied for changes in the endothelium and the PAS-positive basement membrane structure without prior knowledge of the reason for amputation. An attempt also has been made to correlate the distribution of these lesions with certain clinical manifestations of the vascular complications.

MATERIALS AND METHODS

The paraffin blocks of 152 amputation specimens in the files of the Jewish Hospital were collected. These varied in extent from midthigh amputations to the amputation of a single toe. The intensity of the arteriosclerotic process of the main arterial trunks (cognate system) was graded from one to four plus on the basis of the degree of narrowing of the lumen and the intensity of calcification. Large and small vessels were studied primarily for evidence of endothelial proliferation, alterations of elastic structures, and changes in polysaccharide-containing components. As stated, these were placed in groups, as shown in table 1, without prior knowledge of the clinical history, on the assumption that only those cases with diabetes would show a combination of endothelial hypertrophy and hyperplasia and changes in the polysaccharide-containing structures. A comparison was then made with the clinical history and the data tabulated, as presented in table 1.

Successive sections from each paraffin block were stained with hematoxylin-eosin; by the Periodic Acid-Schiff method and by the colloidal iron technic of Rinehart and Abul-Haj[11] for mucopolysaccharide; and by the Verhoeff-van Gieson technic for the demonstration of alterations in elastic fiber, connective tissue and muscle. A fifth section in the series was subjected to microincineration for the in situ demonstration of minerals.

RESULTS

General clinico-pathologic correlation. It is evident from the data in table 1 that we are dealing with a causally related lesion. In the over-all there were three diagnostic errors in the 152 specimens (2.0 per cent); if one considers only the first three disease categories, the error is increased to 2.5 per cent. In the two cases of arteriosclerosis obliterans with lesions similar to those seen in the cases with diabetes, one subject had two fasting blood sugar determinations, which were normal, and the second had a single fasting normal blood sugar. The patient with thromboangiitis obliterans showed a progressively increasing fasting blood sugar from about 85 mg. per 100 ml. several years before amputation, to 138 mg. per 100 ml. at about the time of amputation; this subject therefore may have been diabetic, although such a diagnosis did not appear in the record. In general, the vascular lesions in these three cases were considerably less marked than those seen in most of the diabetics.

There was nothing unexpected about the age distribution shown in various disease categories. Patients with only arteriosclerosis obliterans showed an average age of about nine years older than that found in the diabetic group. The age distribution of the patients with Buerger's disease and idiopathic thrombosis was consistent with general experience. The wider age span in patients with sarcoma of the lower extremity is due to the not uncommon occurrence of such malignancy in younger individuals. The cases with embolism were instances in which the source of the emboli was a mural thrombus of the left ventricle due to myocardial infarction, or of the

TABLE 1

Frequency of PAS-positive, colloidal iron-negative endarteritis in amputated extremities

Disease group	Average age (years)	Number with endarteritis	Number without endarteritis	Total	Per cent positive
Diabetes mellitus	62.7 (44-83)*	85	7	92	92.4
Arteriosclerosis obliterans	71.8 (50-84)*	2	23	25	8.0
Thromboangiitis obliterans	45.4 (30-53)*	1	4	5	20.0
Osteogenic and soft tissue sarcoma	40.6 (13-85)*	0	9	9	0
Traumatic	32.0 (19-61)*	0	7	7	0
Idiopathic thrombosis	43.5 (42-45)*	0	2	2	0
Embolism	64.4 (42-87)*	0	8	8	0
Miscellaneous	52.3 (42-73)*	0	4	4	0

Total cases: 152. Total diabetics: 92. Total nondiabetics: 60.
*Age range.

left atrium in rheumatic heart disease, or a mural thrombus in an arteriosclerotic aorta. The cases in the miscellaneous group received amputations because of extensive trophic ulceration which could not be controlled in patients with disease of the central nervous system, except in one subject in whom amputation was for a hammer toe.

Pathologic characteristics of the vascular lesions. The artery of the largest size in which the "diabetic" lesion could be clearly distinguished was the digital. Here the most prominent feature was an endothelial proliferation of such magnitude as to occlude, almost completely, the lumen in several specimens (figure 1). Characteristically, the internal elastic lamella consisted of a single, intact wavy membrane, in contrast to ordinary arteriosclerosis in which there was usually a duplication and fraying of the elastica. The cellularity of the mass impinging on the lumen was also in contrast to arteriosclerosis, in which the intimal reaction consisted in the deposition of an acellular, hyaline material covered on its internal surface by only a single layer of flattened endothelium. The media in the diabetic lesion was essentially without change, whereas in the arteriosclerotic lesion of the digital artery muscular hypertrophy or hyalinization was sometimes found. The adventitia in the diabetic state and in arteriosclerosis was generally without significant change. With the PAS stain numerous small fibrillae were seen in the vessels of diabetic subjects, forming a reticulated pattern between endothelial cells, and parallel bands of similarly staining material were seen through the media (figure 2). **(See color page 271 for figures 2-5.)** This material did not bind the colloidal iron when the Rinehart-Abul-Haj[11] stain was used (figure 5). When calcification of the media of arteriosclerotic vessels was seen, such foci were generally PAS- as well as colloidal iron-positive, but the "diabetic" lesion showed no tendency towards calcification (figure 6). An interesting additional observation was that in many of the cases of diabetes the elastic lamellae were solidly PAS positive (figure 7), whereas in nondiabetics this structure showed only an outer PAS-positive coating with a clear, unstained matrix.

FIG. 1. Digital artery, diabetic patient. Magnification approx. 250 times, Verhoeff-van Gieson stain. Section shows marked endothelial proliferation with narrowing of the lumen. The internal elastic lamella is a single intact wavy band.

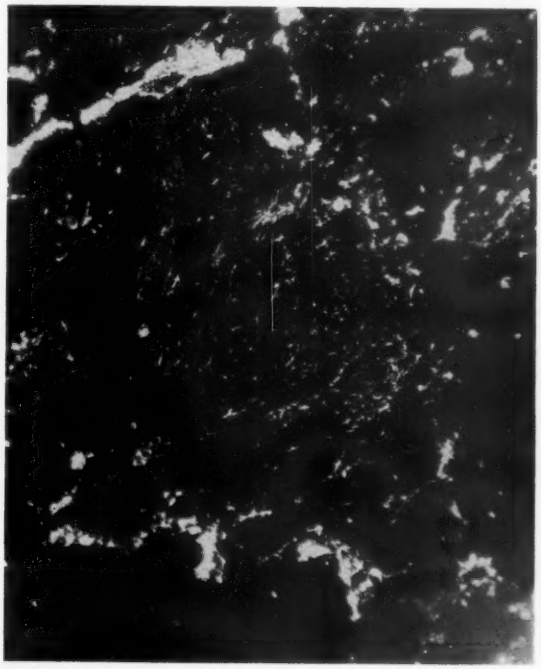

FIG. 6. The same digital artery as in figures 1, 2 and 3. Magnification approximately 250 times, microincinerated section. Note low mineral content of the diabetic vascular lesion.

Since arteriosclerosis of the larger arteries was prevalent in both diabetics and nondiabetics in this series, a comparison of the intensity of the arteriosclerotic process was made, using an arbitrary grading system of one to

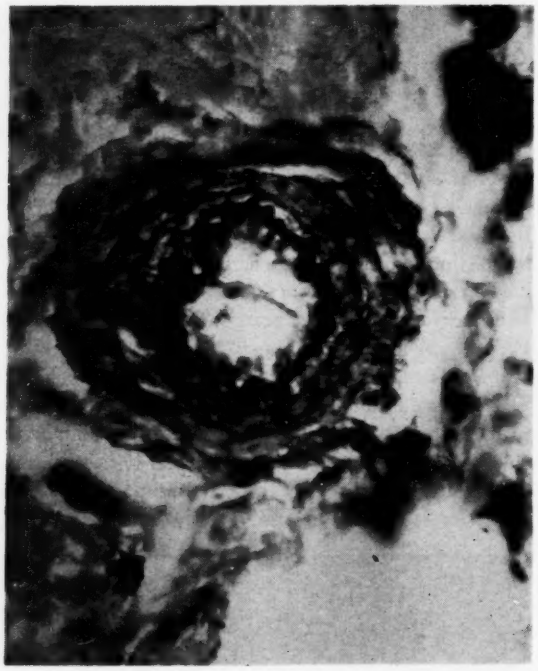

FIG. 7. Periadventitial artery, diabetic patient. Magnification approximately 250 times, Periodic Acid-Schiff stain. Note intense staining of the internal elastic lamella, with the PAS-positive material occupying the position of the matrix of the elastic fiber. This type of staining reaction is often seen in diabetic vascular disease. Note also enlargement of endothelium to a cuboidal form.

FIG. 8. Femoral artery branch, diabetic patient. Magnification approximately 100 times, Periodic Acid-Schiff stain. There is marked endothelial cell proliferation to the point of almost completely occluding the lumen. The internal elastic lamella remains as a single band, solidly PAS positive, and fine PAS fibrils are interlaced through the endothelial cells.

three plus. There was no significant difference in the intensity of arteriosclerosis of the cognate system in these two groups. A similar comparison of calcification in both large and small vessels was carried out, utilizing the micro-incinerated sections, and again no significant difference was found. A significant degree of calcification of small arteries and arterioles was found only in subjects with long-standing hypertension.

In the smaller arteries of diabetics various stages of the lesion could be traced. The initial change appeared to be marked enlargement of endothelial cells, followed by their proliferation, sometimes with almost complete occlusion of the lumen (figure 8). Again the internal elastic lamellae often stained solidly with the PAS technic, and PAS-positive, colloidal iron-negative fibrils were scattered in a network through the layer of proliferating endothelium.

At the arteriolar and capillary level the fibrillary changes were more striking than the endothelial proliferation. Reduplicated or split fibrils formed concentric rings, which were again PAS positive and colloidal iron negative (figure 9). Within the spaces between these fibrils and lining the lumen, enlarged endothelial cells were found. Not only were such lesions observed in the vascular supply to various structures, as noted below, but they could be found in recanalized lumina of previously thrombosed arteries (figure 10), whereas they were absent in the recanalization process in arteriosclerosis obliterans and Buerger's disease. In diabetics they often progressed to the formation of solid hyaline masses, which again were PAS positive and colloidal iron negative. A similar progression of such lesions of small arteries, arterioles and capillaries was also found in nerves, the first and last stages of which are shown in figures 11 and 12.

Because of the almost consistent pattern of PAS-positive, colloidal iron-negative reaction in the fibrillae in these lesions (a colloidal iron-positive reaction was sometimes seen, in addition, in the muscular vessels of patients with diabetes and hypertension), it was thought that additional support for the specificity of this lesion could be obtained if it could be shown that the glomerular

FIG. 9. Arterioles in muscle, diabetic patient. Magnification approximately 250 times, Periodic Acid-Schiff stain. There are concentric rings of PAS-positive material between which an occasional enlarged endothelial cell can be detected. Note also prominence of capillaries.

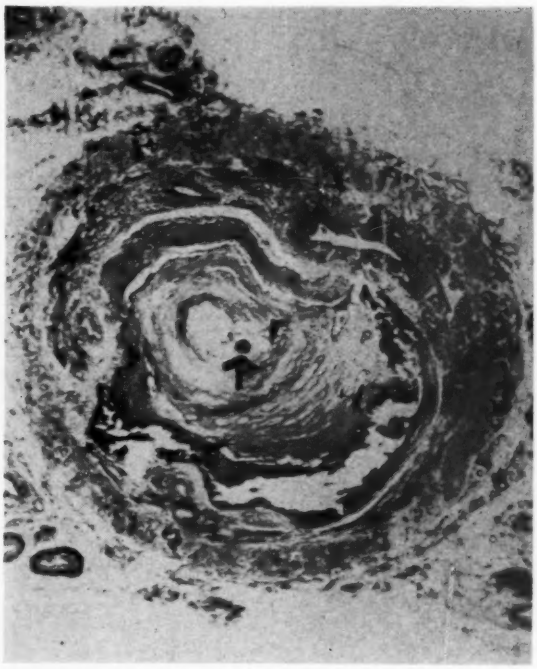

FIG. 10. Anterior tibial artery, diabetic patient. Magnification approximately forty times. The lumen is occluded by atheromatous reaction except for a tiny opening surrounded by an intensely PAS-positive ring (arrow). This ring failed to take up colloidal iron.

lesions of diabetes showed a similar staining response. Accordingly, sections from the kidneys of eight cases with diabetic glomerulosclerosis as well as cases with essential and malignant hypertension were stained with these two technics. The hyaline material in all of the glomeruli showing the lesions of diabetic glomerulosclerosis was, without exception, PAS positive and colloidal iron negative (figures 3 and 4, **page 271**). On the other hand, the lesions of small arteries in the kidneys of patients with benign or malignant hypertension were colloidal iron positive.

Frequency distribution of the "diabetic" vascular lesion. Vascular lesions of the type described in the foregoing section were found in the adventitial tissues about vessels of the major arterial trunks, in septal vessels of muscle, in the vascular supply to nerves, and in the dermal vessels of the skin. Unfortunately, a representation of all of these structures was not available in each specimen, so that the data shown in table 2 are based on the number of specimens obtained in each category. Lesions were found in the vasa vasorum of almost every diabetic extremity, in the nerves of almost three fourths of the cases, in the dermal vessels of over 60 per cent, and in the small vessels supplying the muscles in slightly less than one half of the available specimens.

Correlation with diabetic neuropathy. Because of the frequency with which vascular changes were found in the nerves, it was decided to attempt a correlation of the presence or absence of these "diabetic" lesions with clinical evidence of diabetic neuropathy. This correlation is shown in table 3, where it can be seen that vascular lesions were found in all but two of the cases with clinical neuropathy in which nerve specimens were available. In those cases in which the clinical evidence of nerve involvement was questionable, about half showed pathologic alterations in vessels. Only one case showed vascular involvement of nerves without clinical evidence of neuropathy.

Frequency of hypertension in diabetic gangrene. About two thirds of the diabetic cases with gangrene were hypertensive as compared with less than half of the nondiabetics who had undergone an amputation of the extremity. Among the diabetics hypertension was about equally distributed between the two sexes, while in the

FIG. 11. Neural arteriole, diabetic patient. Magnification approximately 500 times, hematoxylin-eosin stain. Note marked hypertrophy of endothelial cells.

FIG. 12. Neural arteriole, diabetic patient. Magnification approximately 500 times, Verhoeff-van Gieson stain. Note complete obliteration of the vessel. A few endothelial nuclei remain at the periphery.

TABLE 2

Frequency distribution of vascular lesions in various sites in diabetes cases

Site	Number of specimens available	Number with vascular lesions		Per cent
Vasa vasorum	78	76		97.4
Vasa nervorum	66	48		72.7
Dermal vessels	51	32		62.7
Muscular vessels	59	28		47.5
Total	254	184	Average	72.4

TABLE 3

Clinico-pathologic correlation of diabetic neuropathy

	Clinical findings	Pathologic findings Positive lesions	No lesions
Number of cases with neuropathy	24	22	2
Number of cases with questionable neuropathy	13	6	7
Number of cases without evidence of neuropathy	8	1	7

nondiabetics there was a predominance of females. Since almost one third of the diabetic patients with gangrene were not hypertensive, it is evident that hypertension is not a necessary etiologic component of the "diabetic" vascular lesion of the lower extremity (table 4).

Comparison of the frequency of palpable pulses in arteries of diabetic and nondiabetic patients. With the possible exception of the diabetic group with gangrene, the number of cases is too few to permit a significant comparison of the frequency of such palpable pulses in various diseases afflicting the lower extremity (table 5). Despite the large margin for error with this diagnostic technic, it is perhaps significant that in one third of the diabetic cases with gangrene there were palpable pulses at all of the common points of palpation, and in 10 per cent such pulses were as strong as on the unaffected side. This, along with the evidence below, of the patchy distribution of gangrene more commonly found in diabetic patients, would appear to indicate that in the latter disease gangrene may frequently occur without complete occlusion of one of the main arterial trunks.

Comparison of gangrenous involvement in diabetic and nondiabetic patients. In table 6 is shown a comparison

TABLE 4

Frequency of hypertension in diabetic and nondiabetic groups

Diabetic				Nondiabetic			
Hypertensive		Nonhypertensive		Hypertensive		Nonhypertensive	
Male	Female	Male	Female	Male	Female	Male	Female
19	22	11	9	9	5	14	5
63.3%	74.2%	36.7%	25.8%	39.1%	50%	60.9%	50%
67.2%		32.8%		42.4%		56.6%	

TABLE 5

Frequency of palpable pulses in various disease groups

	Pulse characteristics						
	Strong		Weak		Absent		Total
Disease group	Number	Per cent	Number	Per cent	Number	Per cent	
Diabetes mellitus	6	(10)	14	(23)	40	(67)	80
Arteriosclerosis obliterans	0		3	(33)	6	(67)	9
Thromboangiitis obliterans	0		0		2	(100)	2
Embolism	0		0		7	(100)	7
Idiopathic thrombosis	1	(50)	0		1	(50)	2

TABLE 6

Comparison of distribution of gangrene in diabetic and nondiabetic patients

	Diabetic		Nondiabetic	
Site of involvement	Number of cases	Per cent	Number of cases	Per cent
Midthigh to toes	1	0.9	5	12.5
Knee to toes	2	1.8	9	22.5
Midtibial to toes	3	2.7	6	15.0
Patchy areas on foreleg	2	1.8	0	—
Ankles to toes	29	26.1	13	32.5
Toes only	41	36.9	6	15.0
Patchy areas on foot	33	29.7	1	2.5
Total	111		40	

of the distribution of areas of gangrene in diabetic and nondiabetic subjects. This tabulation includes all cases in which clinical information was available, hence it includes all of the cases in which tissue was available for study as well as those in which pathologic specimens were not obtained. It is evident that gangrene involving areas above the ankle was present in only about 7 per cent of the diabetic group, as compared with almost 50 per cent in the nondiabetic group. In fact, only about 5 per cent of the diabetic group showed massive gangrene, with a sharp demarcation line above the level of the ankle, as compared with about half of the nondiabetics. A sharply demarcated zone of gangrene, involving all of the foot, occurred with almost equal frequency in the two groups (26.1 per cent in diabetics and 32.5 per cent in nondiabetics). Patchy areas of gangrene of the skin (most often of the foot), or involvement of one or several toes constituted the pattern of involvement in almost 70 per cent of the diabetics as compared with only 17.5 per cent in nondiabetics.

It is apparent, then, that massive gangrene of the lower extremity with demarcation at about the midthigh, knee or midtibial region is more characteristic of peripheral vascular disease in nondiabetics, while patchy areas of gangrene of the skin or involvement only of one or several toes is more characteristically found in diabetes. However, sharply demarcated gangrene involving all or most of the foot is about equal in the two groups.

Correlation of gangrene of lower extremity with diabetic retinopathy, nephropathy and the administration of insulin. In only sixty-two of the diabetic patients with gangrene of the lower extremity was there sufficient information in the clinical history with which to correlate the associated frequency of retinopathy and nephropathy, and to determine whether or not insulin had ever been administered prior to the onset of gangrene. The data on such correlations are shown in table 7. While the frequencies of these two complications are almost identical, this is merely coincidental, since many of the cases had only one or the other. This evaluation of retinopathy and nephropathy has been made solely on clinical grounds, since only four of these patients came to autopsy.

TABLE 7

Frequency of retinopathy, nephropathy and insulin therapy in sixty-two diabetic patients with gangrene

	Present		Questionable		Absent		
	Number	Per cent	Number	Per cent	Number	Per cent	Total
Retinopathy	11	17.7	21	33.9	30	48.4	62
Nephropathy	11	17.7	22	35.5	29	46.7	62

Insulin	Received prior to amputation		Not received prior to amputation		
	Number	Per cent	Number	Per cent	Total
	39	62.9	23	37.1	62

Interestingly, in slightly less than half of the cases there was no clinical evidence of either of these two vascular complications of diabetes. Retinopathy and nephropathy were diagnosed clinically in 17.7 per cent of the cases, and there was a strong index of suspicion in about one third of the patients. Thus, if one combines the instances of a positive and a questionable diagnosis, it would appear that in only slightly more than half of the cases could there have been associated retinopathy and nephropathy on clinical grounds.

In twenty-six of the cases with diabetes the patient received no insulin prior to amputation. Significantly, in the majority of these the diabetes was considered so mild prior to the onset of gangrene that it was considered to be controlled without the administration of insulin. Yet these patients developed gangrene of the lower extremity; six had definite nephropathy and in another six it was questionable. Thus it would appear that all of these vascular complications may occur in patients considered to have clinically mild, well-controlled diabetes.

DISCUSSION

It is evident that gangrene of the lower extremity is considerably more common in diabetics than in nondiabetics (Dry and Hines;[12] Lisa et al.;[13] Bell;[14] Lundbaek[15]). In Bell's extensive series the ratio of diabetics to nondiabetics was about forty to one. Joslin et al.[16] have expressed the belief that atherosclerosis (lipid plaque formation) is found primarily in elastic type arteries in the nondiabetic, but is a common occurrence in the muscular arteries of the legs of diabetic patients. While Wilder[17] also accepts the importance of atherosclerosis in the diabetic, he differs from Joslin et al.[16] in that he believes that atherosclerosis may be found also in the muscular arteries of nondiabetics. On the other hand, Lisa et al.[13] have found that atherosclerosis may be mild or severe in either diabetic or nondiabetic gangrene. They are in agreement with our view that it is only in the severe cases of either type of gangrene that the dorsalis pedis artery is involved, and presumably would also agree that the digital arteries rarely show significant sclerosis even when there is advanced disease of the larger muscular arteries. As in the present study Lisa et al.[13] did not find a distinguishable difference in the intensity of arteriosclerosis between the amputated extremities of diabetic and nondiabetic patients with gangrene. This is also supported by LeCompte's[18] statement that "as far as we know, there is no qualitative difference in diseases of the larger arteries between diabetics and nondiabetics."

As Warren and LeCompte[19] have pointed out, when there is progressive narrowing of the lumen of an artery, there is time for collateral circulation to develop. While there may be pain and disability during the process of readjustment, eventually a delicate point of equilibrium is reached where the combined blood supply from both cognate system vessels and collaterals is just sufficient for the ordinary needs of the limb. Any unusual stress may tip the balance. In the final analysis then, the state of the smaller arteries, upon which the establishment of a collateral supply is dependent, is the principal determining factor. It is in this regard that the present findings are of particular significance. McKittrick and Root[20] have been able to demonstrate instances of failure of the establishment of an adequate collateral circulation by the intra-arterial injection of amputated extremities and have pointed out that such a failure often exists in diabetic gangrene. The narrowing and closure of small arteries in diabetic patients with already advanced arteriosclerosis of cognate vessels, by lesions such as have been described here, could account for the failure to develop an adequate collateral circulation; it could also account for the increased frequency of gangrene in diabetes.

LeCompte[21] and others hold the opinion that atherosclerosis, Mönckeberg sclerosis and arteriolar sclerosis all appear to be quantitatively increased in diabetes, and presumably this would account for the increased frequency of gangrene. However, as we have recently pointed out,[22] certain reservations are in order with respect to such a conclusion. In a series of studies dealing with vessels in a number of anatomic sites, Blumenthal and co-workers[23-26] have been unable to demonstrate any increase in the intensity of arteriosclerosis of major arteries in diabetic patients when the factors of age and coexistent hypertension were properly assessed in the comparison. Studies by Wilens[27] and by Dible[28] also support the latter conclusion.

Since the classical paper of Moritz and Oldt[19] it has been recognized that the commonest lesion of small arteries and arterioles (arteriolosclerosis) consists of

intimal fibrosis with medial hypertrophy and hyalinization. This lesion is most often associated with hypertension, but is seen only occasionally in the absence of hypertension. The so-called hyperplastic intimal change in this lesion consists of concentric laminated layers of fibrous tissue in the intima, often with reduplication of the internal elastic lamella. It thus resembles the diffuse intimal thickening or early plaque formation of larger arteries, and like the latter, according to Rinehart and Abul-Haj,[11] contains abundant colloidal iron-positive material. The medial coat in arteriolosclerosis undergoes hypertrophy, and Muirhead et al.[30] have shown that when this coat gives a PAS-positive reaction it is due to degenerative changes in muscle fibers. Presumably in the latter event the material in the muscular layer would also be colloidal iron positive, and in preliminary observations on the small arteries in malignant nephrosclerosis in our laboratories this appears to be the case. It is pertinent to the foregoing that about one third of the patients with diabetic gangrene were not hypertensive, and Allen, Barker and Hines[31] have pointed out that it is not unusual to find gangrene due to occlusive disease of the arteries of the lower extremity in normotensive subjects.

It would thus appear that the lesions described herein are not a variant of the arterio-arteriolosclerosis associated with hypertension. The endothelial proliferation which is such a prominent component of the "diabetic" lesion is not a component of the lesion of arterio-arteriolosclerosis. The cellular intima which develops as a result of this proliferative activity is in contrast to the fibrous or hyaline character of arterio-arteriolosclerosis. Furthermore, the internal elastic lamella of the "diabetic" lesion characteristically remains intact, whereas in the sclerotic lesion it frequently undergoes fraying and reduplication. The laminated layers of the intimal lesion are PAS positive and colloidal iron negative in diabetes, whereas in arterio-arteriolosclerosis the PAS reaction is variable and the colloidal iron technics may give a Prussian blue color. As to medial changes, atrophy is seen more often than medial hypertrophy in the "diabetic" lesion, as this layer becomes incorporated in the expansion of the concentric laminated layers.

It would also appear that the vascular lesions described herein have in common with the specific glomerular lesion of diabetes, as described in electron microscopy studies by Bergstrand and Bucht,[6] as well as Irvine et al.[7] and Rinehart et al.,[9] an endothelial hypertrophy and hyperplasia and an increase in endothelial basement membrane substance. They also have in common with the specific diabetic glomerular lesion the colloidal iron-negative characteristic of endothelially derived basement membrane substance as described by Rinehart et al.[8] The "diabetic" lesion in arteries of the lower extremity differs from the glomerular lesion in that in the former the PAS-positive material is usually laid down in a fine reticulated pattern, whereas in the latter it is laid down in thick prominent masses. However, in some of the vascular lesions seen in nerves, the end stage consisted of large masses similar to those seen in the glomerulus (figure 12).

Certain other observations tend to support the present findings. Thus Ditzel[32,33] has reported changes in the conjunctival vessels characteristic not only of diabetic patients but also of their relatives and children. Mendlowitz et al.[34] have observed a decrease in hallucal circulation and Megibow et al.[35] have reported occlusive disease of the digital circulation in young diabetics. The latter studies, while unconfirmed by microscopic observations, suggest that disease of small vessels may already be advanced in diabetics while there is still no evidence of an insufficiency of large arteries. Some years ago Woltman and Wilder[36] attributed the neuropathy of diabetes to vascular changes which they described as a "thickening of the walls of the intraneural vessels." More recently Fagerberg[37] presented a series of studies in which he showed that diabetic neuropathy is also associated with vascular lesions of nerves, and in the group over fifty years of age practically all cases of neuropathy were combined with retinopathy, nephropathy, or some other evidence of vascular disease. However, he failed to distinguish these lesions on a pathologic basis. More directly related is the observation of Burstein, Blumenthal and Soule[10] of vascular lesions in the placentas of diabetic mothers comparable to those described here.

Blumenthal[38] has pointed out that the arteries have a limited response potential, i.e., only a limited number of ways in which they are capable of responding to a large variety of injurious influences. The arterio- and arteriolosclerotic processes described above represent one such type of response, which occurs most often in response to physical (hemodynamic) forces, but which also may occur in response to the infiltration of lipids or to other injurious factors. A lesion of the type described herein in association with diabetes and characterized primarily by endothelial proliferation represents a different type of response. Such endothelial proliferation is seen in certain infectious diseases afflicting arteries, such as syphilis, but the "diabetic" lesion lacks the inflammatory component generally present in infections of arteries. A similar endothelial response may occur on an immuno-

allergic basis as in Buerger's disease, and probably in other as yet poorly understood conditions. The "diabetic" lesion lacks the component of thrombus formation present in thromboangiitis obliterans. Nevertheless, because of such considerations, one must reserve judgment as to the limits of specificity. It would appear, however, that the lesions herein described, in all likelihood, have a causal relation to diabetes.

SUMMARY

A study of the small vessels of 152 amputation specimens of the lower extremity is herein reported. Ninety-two of these specimens were in diabetic patients and the remaining sixty in patients with a variety of other conditions necessitating amputation. A lesion of small arteries and arterioles of diabetic patients has been described, which appears to have a causal relation to diabetes. These lesions can be distinguished from arterio-arteriolosclerosis by certain pathologic characteristics and by histochemical technics; they are characterized by endothelial proliferation and the deposition of a PAS-positive material in a reticulated pattern which is colloidal iron negative. By contrast, the lesions of arterio- and arteriolosclerosis are characterized by the deposition of a fibrous hyaline acellular material, as well as of a fraying and reduplication of the internal elastic lamella; the latter sometimes contains a PAS-positive material which is usually also colloidal iron positive. Furthermore, about one third of the cases with diabetic gangrene and the "diabetic" lesion were normotensive.

The vascular lesions believed to be causally related to diabetes have been found in the vasa vasorum and periadventitial vessels of cognate system arteries, as well as the small arteries and arterioles of nerves, muscles and skin. They have been found in diabetic patients without evidence of hypertension and in some who had not received insulin prior to amputation.

Clinical correlations have been carried out with respect to the manifestations of neuropathy, retinopathy and nephropathy, as well as to pulse characteristics. It has been shown that massive gangrene of the lower extremity is more characteristic of the nondiabetic patient with occlusive vascular disease, while a patchy distribution is more characteristically found in the diabetic. With respect to neuropathy, vascular lesions were found in all but two patients with definite clinical neuropathy, in half of the cases in which neuropathy was questionable, and in only one case without clinical evidence of involvement of peripheral nerves. In one third of the patients with diabetic gangrene there were palpable pulses at all of the common points of palpation, and in 10 per cent such pulses were as strong as on the unaffected side.

SUMMARIO IN INTERLINGUA

Morbo Periphero-Vascular Nonatheromatose In Le Extremitates Inferior De Patientes Con Diabete Mellite

Es reportate un studio del vasos minor in 152 specimens de amputation de un extremitate inferior. Novanta-duo del specimens veniva ab patientes con diabete, le remanente sexanta ab patientes con un varietate de conditiones requirente le amputation. Es describite un lesion del micre arterias e arteriolas in patientes diabetic, que ha apparentemente un relation de effecto a causa con le disordine diabetic. Iste lesiones pote esser distinguite ab arterio-arteriolosclerosis per certe characteristicas pathologic e per medio de technicas histochimic. Illos se distingue per proliferation endothelial e le deposition de un substantia positive a PAS in un configuration reticular que es negative a ferro colloide. Per contrasto, le lesiones de arterio- e de arteriolosclerosis es characterisate per le deposition de un substantia acellular hyalin fibrose e etiam per un reduplication del interne lamella elastic. Iste lamella contine un material que es positive a PAS e usualmente etiam a ferro colloidal. In plus, circa un tertio del casos con gangrena diabetic e le lesion "diabetic" esseva normotensive.

Le lesiones vascular que nos crede causalmente relationate a diabete esseva trovate in le vasa vasorum e le vasos periadventitial de arterias de systema cognate e etiam in le micre arterias e arteriolas de nervo, musculo, e pelle. Illos esseva incontrate in patientes diabetic sin manifestation patente de hypertension e in certe patientes qui habeva recipite nulle insulina ante le amputation.

Correlationes clinic esseva effectuate con respecto al manifestationes de neuropathia, retinopathia, e nephropathia e etiam con respecto al characteristicas del pulso. Esseva monstrate que un gangrena massive del extremitates inferior es plus characteristic del patiente non-diabetic con morbo vascular occlusive, durante que un distribution disperse se incontra plus characteristicamente in le diabetico. Quanto al neuropathia, lesiones vascular esseva trovate in omne excepte duo patientes con definite neuropathia clinic, in un medietate del casos in que neuropathia esseva questionabile, e in solmente un caso sin manifestationes clinic de un affection de nervos peripheric. In un tertio del patientes con gangrena diabetic, pulsos palpabile esseva presente a omne le punctos commun de palpation, e in 10 pro cento del casos tal pulsos esseva tanto forte como al latere non-afficite.

ACKNOWLEDGMENT

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FIG. 2. The same digital artery as in figure 1. Magnification approximately 250 times, Periodic Acid-Schiff stain. Note the extensive interlacing network of PAS-positive fibrils between endothelial cells.

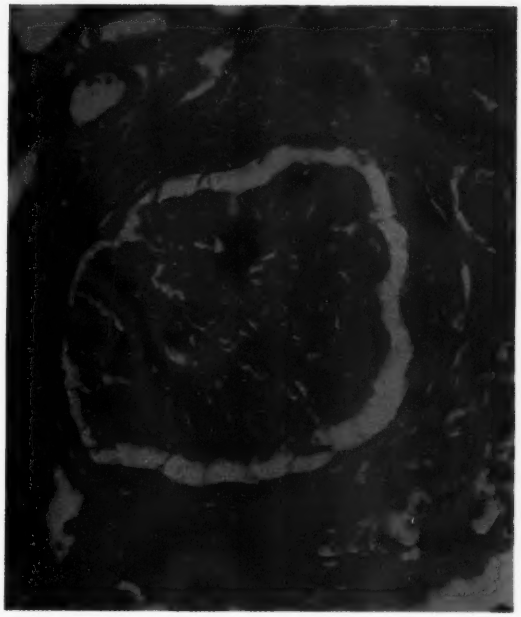

FIG. 3. Glomerulus, diabetic patient. Magnification approximately 250 times, Periodic Acid-Schiff stain. Note nodular masses of PAS-positive material in the capillary tufts.

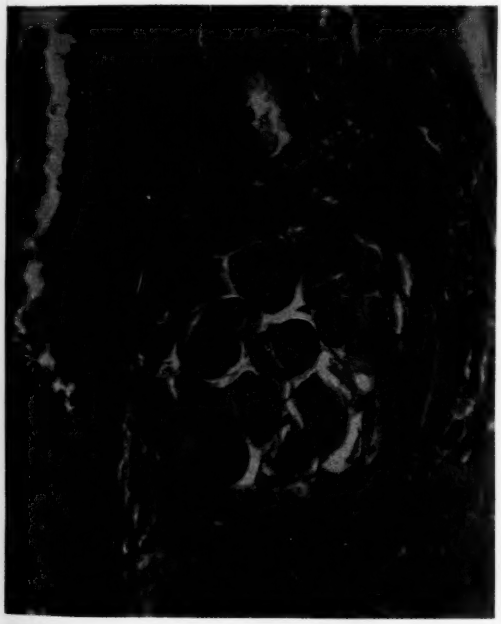

FIG. 4. Glomerulus, same diabetic patient as in figure 3. Magnification approximately 250 times, colloidal iron stain of Rinehart and Abul-Haj. Nodular masses stain an olive green, and thus fail to take up colloidal iron. Adjacent vessel showing minimal sclerosis contains blue colloidal iron-positive material.

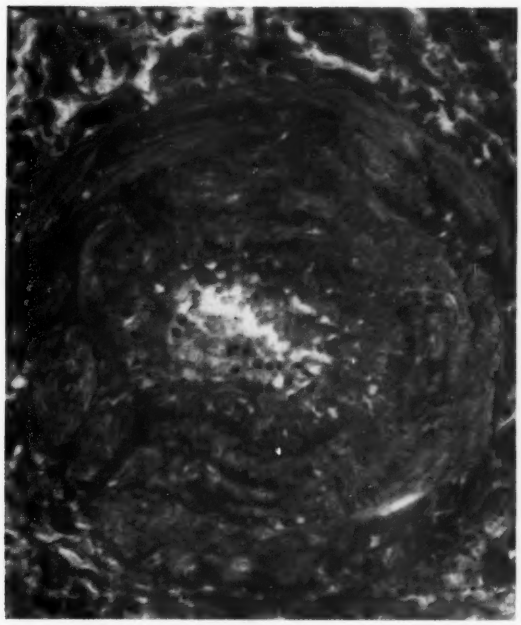

FIG. 5. The same digital artery as in figures 1 and 2. Magnification approximately 250 times, colloidal iron stain of Rinehart and Abul-Haj. The zone of endothelial proliferation fails to take the colloidal iron stain.

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Metabolic and Endocrine Studies with Phenethyldiguanide (DBI)

Thomas G. Skillman, M.D., Fred A. Kruger, M.D., and George J. Hamwi, M.D., Columbus

The recent report by Ungar and his colleagues[1] describing the hypoglycemic action of a nonsulfonamide compound has resulted in widespread clinical investigation of its possible use as an oral agent for the treatment of diabetes mellitus. This compound is the hydrochloride of phenethyldiguanide (N-beta-phenethylformamidinyliminourea hydrochloride) and has been designated DBI by the manufacturer and hereafter will be referred to as such. Although the mechanism of the hypoglycemic effect of the sulfonylurea compounds is not completely understood, it appears likely that it is predominantly an insulin effect, and that they act by stimulating the beta cells to secrete insulin. The hypoglycemic action of DBI does not appear to be mediated by insulin.

Since 1957, many investigators[2-6] as well as ourselves have conducted studies to assay the therapeutic efficacy and possible untoward effects of this compound in diabetics. Our experience with DBI has been gained from evaluation of hospitalized diabetic patients who presented specific problems in management. Particular emphasis was placed upon evaluation of the effects of the drug on thyroid, adrenal, hepatic, renal, gastrointestinal, and hematopoietic function as well as its influence upon the diabetic state. Studies also have been initiated to explore the mechanism of action of this type of compound.

Our findings, to date, indicate that DBI does not alter glandular physiology as reflected by the customary indices, and aside from its gastrointestinal side effects, does not influence the function of vital organs in an untoward fashion. DBI has been employed as the sole therapeutic agent to control hyperglycemia and glycosuria in the milder, obese diabetic whose disease is of short duration.

Presented at the Annual National Meeting of the American Federation for Clinical Research in Atlantic City, New Jersey, on May 4, 1958.

From the Division of Endocrinology, Department of Medicine, Ohio State University, Columbus, Ohio.

METHODS

During a twelve-month period, twenty-nine selected diabetic patients were hospitalized on the metabolism service with the sole aim of evaluating their response to DBI. In each patient, control of hyperglycemia and glycosuria was not optimal prior to admission, either because the patient was a recently discovered diabetic, or because preadmission treatment with tolbutamide or insulin and diet presented problems in diabetes management.

A uniform plan of observation for evaluating the influence of DBI on hyperglycemia and glycosuria, endocrine function and vital organ function was adopted for all patients. A forty-eight- or seventy-two-hour control period preceded treatment with DBI in each instance. During this interval a diet believed optimal for control of diabetes was fed and the same diet was continued during the treatment period. Recently discovered diabetics and those not taking insulin were observed while treated with diet alone. In those patients who took insulin prior to study, the customary dose was continued during this period and then diminished or deleted during DBI therapy. Determinations of various functions made during the control period were then compared with similar findings made during the DBI treatment period. Daily fasting and two-hour postprandial blood sugar levels as well as twenty-four-hour urinalyses for volume and quantitative sugar were used to estimate the effect of the drug on carbohydrate metabolism. Paired values for serum cholesterol, protein bound iodine, and twenty-four-hour thyroidal I^{131} uptake were used to assay thyroid function. Estimates of adrenal physiology were made by daily twenty-four-hour determinations of urinary 17-ketosteroid, 17-hydroxycorticoid and creatinine excretion. Evaluation of liver function included cephalin flocculation, bromsulfalein retention, prothrombin concentration, serum alkaline phosphatase content and serum glutamic-oxaloacetic transaminase tests. Other indices included hemoglobin, hematocrit, white blood cell and differential counts. Blood urea

nitrogen measurements and total urinary protein determinations were also performed.

DBI was administered orally with meals. Considerable variation in dose and in the timing of dosage occurred and was incident to attempts to avoid gastrointestinal side effects of the drug and to improve control of hyperglycemia. DBI was given for at least three days to all subjects and always was continued until a clear-cut appraisal of hypoglycemic response could be made. The criteria for response were rigid and included: (1) ability to stop insulin; (2) a drop in blood sugar resulting in levels consistently below 175 mg. per cent including postprandial values; (3) a drop in glycosuria to less than 5 gm. per day; and (4) tolerance to the drug of such degree that gastrointestinal effects were in no fashion disturbing to the patient.

Most of the patients who met these criteria for response were then discharged from the hospital and followed at monthly intervals as outpatients. The study of all indices was continued.

RESULTS AND COMMENT

Hypoglycemic action. In twenty-nine instances an attempt was made to employ DBI as the sole hypoglycemic agent. In all instances fasting blood sugars exceeded 175 mg. per cent during the control period despite the administration of a carefully planned diet. In sixteen individuals insulin in suboptimal doses also was used during the control period.

Fourteen met the criteria for adequate hypoglycemic response while fifteen failed to do so. In the responsive group the fasting blood sugar levels fell from a mean of 240 mg. per cent during the control period to a mean of 135 mg. per cent during the fourth, fifth, and sixth day of DBI treatment. None of the patients responding received insulin during the treatment period. In the nonresponsive group, fasting blood sugars rose from a mean of 277 mg. per cent during the control period to an average of 350 mg. per cent on the fourth, fifth and sixth days of DBI therapy. These findings are summarized in figure 1.

Age. Only two true juvenile diabetic patients were included in the study, and neither was found to be responsive. One was a ten-year-old girl who had been symptomatic for only a few weeks. Dosage with 150 mg. of DBI daily was without effect, but subsequent insulin therapy in a dose of 20 units and ultimately 10 units daily resulted in excellent control. The other was a twenty-five-year-old man who had been diabetic for fifteen years, and two years prior to DBI treatment had undergone surgical hypophysectomy with the hope

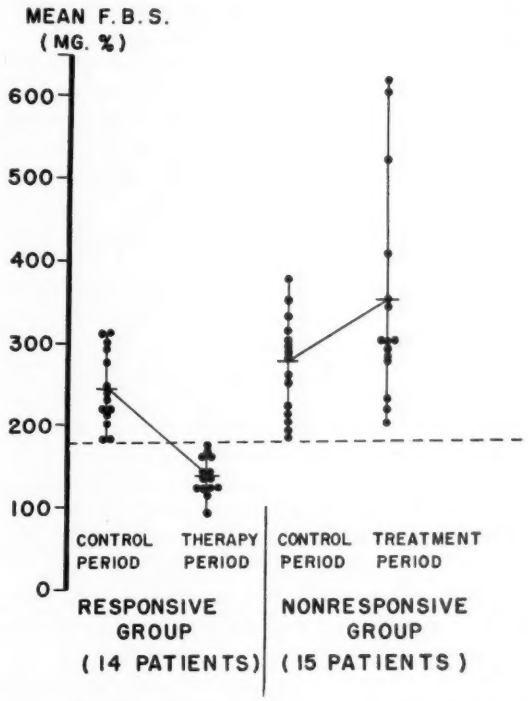

FIG. 1. Comparison of mean fasting blood sugar values measured during the control period and during the fourth, fifth and sixth days of therapy in the responsive and nonresponsive groups.

that his severe progressive retinitis proliferans might be controlled. This operation resulted in a decrease of his daily insulin requirement from 80 to 14 units per day. When DBI therapy was instituted, his NPH insulin dose was arbitrarily decreased from 14 to 8 units; but over a three-day period, his fasting blood sugar rose from 130 to 620 mg. per cent, and his glycosuria rose from 12 to 68 gm. daily. Aside from these two patients, age correlated poorly with responsiveness: Subjects as young as twenty-two and twenty-seven years of age responded, and those as old as sixty-one or sixty-two failed. The mean age of each group was essentially the same.

Sex. Slightly fewer men than women were studied, but no sex difference was found between those who responded, (eight women and six men), and those who failed, (eight women and seven men).

Duration of diabetes. A high degree of correlation between the duration of the diabetic state and response to DBI was noted. In the responsive group the mean duration of the disease was 1.7 yr. and four subjects were recently discovered diabetics. No patient who had been

diabetic for more than five years responded. The mean duration of the disease in the failure group was five times greater (8.6 yr.). Three of these patients were recently discovered diabetics. In this group persons having been diabetic 25, 20, 15, 12, and 11 yr. gave no evidence of responsiveness to the drug, while a few of those in whom the duration was less than ten years responded, but in a suboptimal fashion.

Insulin dose. With regard to the magnitude of insulin dose, differences between the responsive and nonresponsive groups could also be established. Among those responding, half took insulin prior to the trial of DBI while within the nonresponsive group, three fourths used insulin. The daily dosage of insulin averaged 10.2 U. per patient for all individuals in the responsive group, and averaged 25.3 U. per patient in the nonresponsive group. No patient who used more than 26 U. of insulin responded. It was noted that the insulin requirement could be diminished 10 to 25 per cent among some subjects classified as nonresponsive. However, the daily insulin dose of a fifty-eight-year-old man who had undergone a total pancreatectomy three years prior to study was not altered by the administration of as much as 225 mg. of DBI daily for a week.

Body type. Very definite differences were found when the incidence of overweight patients in each group was determined. If simple height and weight charts were employed to categorize those who were more than 10 per cent overweight, eight of the fourteen patients who responded could be so classified while only one of the fifteen who failed to respond fit these criteria. Although it was true that obese patients tended to respond as a group, body type alone could not be employed as a criterion to predict responsiveness, as certain lean subjects responded well.

History of previous diabetic ketosis. When a history of ketoacidosis was obtained, no patient was found responsive. Two thirds of the subjects within the nonresponsive group gave such a history. The relationship of ketosis to responsiveness was further extended as three nonresponsive subjects developed ketoacidosis during their trial of DBI therapy. One patient, a fifty-one-year old man, became ketotic after four days of therapy when his serum CO_2 content fell to 10 mEq./L. and blood sugar rose to 1,024 mg. per cent.

Magnitude of DBI dose. Considerable individual variation in response to DBI appeared to exist within members of the responsive group. Variations in daily dosage from 100 to 300 mg. were necessary to control different patients whose diabetes seemed equally severe. When blood sugars reached normal levels, it was usually possible to lower the dose so that the customary dose averaged 150 mg. daily. The magnitude of the dose was limited by side effects which were predominantly gastrointestinal and included nausea, anorexia, a metallic taste, and occasionally abdominal cramping, emesis, and diarrhea. Three individuals complained of severe generalized weakness. These reactions usually occurred as the dose approached 225 mg. daily and regressed or disappeared promptly as the dose was decreased or temporarily omitted. Aside from the actual size of the dose of DBI, a definite time factor seemed related to response. Among subjects reacting favorably, none displayed a significant decrease in hyperglycemia until the second treatment day, and none responded optimally until the third day. As a group, these patients required four days to respond; and in one person, normoglycemic levels were not reached until the sixth day. Our criteria for responsiveness were achieved with a mean dose of 195 mg. of DBI daily at this time. The observed delay in hypoglycemic response was also seen in nondiabetic subjects: When a dose of 100 mg. of DBI was given orally, no drop in fasting blood sugar was found in any of six subjects followed for five hours with hourly blood sugar determinations.

Long-term studies. Of the fourteen patients who exhibited optimal response to DBI, nine were followed as outpatients for periods ranging from seven to thirteen months; and the remaining five could not be followed, either because they lived at distances too great from the hospital for intensive sequential study, or because they chose not to take part in this phase of the experimental program. The nine who were followed provided eighty-three patient-months of study. The daily dose averaged 150 mg. Control of hyperglycemia in eight of these patients could be classified as excellent, as each reported total absence of glycosuria during the entire period; and none was found to have a blood sugar exceeding 200 mg. per cent at any time. The remaining patient, who thus may be classified as a secondary DBI failure, was an obese thirty-seven-year-old housewife who was well controlled symptomatically until the seventh month of therapy. At this time, she developed a pelvic infection, and diabetes control deteriorated: DBI was discontinued and treatment with insulin was begun. She now takes 30 U. of NPH insulin daily, but hyperglycemia and glycosuria are still not optimally controlled. DBI treatment was discontinued in one other patient. This subject was a twenty-seven-year-old engineer who was ideally controlled with 150 mg. of DBI daily. Dosage was stopped after seven months to challenge the need for the drug. During five months, since discontinuation

of DBI, blood sugar levels have remained within normal limits and aglycosuria has persisted. It appears most likely that a natural temporary remission of diabetes has occurred.

Careful scrutiny was given to all subjects to elicit signs of possible toxicity to the drug. Mild complaints of a metallic taste sensation, frequent defecation, and intermittent nausea were occasionally registered, but in no instance was withdrawal of the drug necessary. Other complications beset two of these patients, but in neither instance could these events be ascribed to DBI. One patient, who was known to have coronary heart disease, developed congestive failure which responded to the customary measures while DBI was continued, and another suffered from pelvic inflammatory disease. No change in weight of significant magnitude was observed; consequently, if an anorexogenic effect of DBI was operative, it was not sufficient to decrease caloric intake.

STUDIES ON ENDOCRINE AND VITAL ORGAN FUNCTION (SEE TABLE 2)

Thyroid function. Values of thyroid function obtained during treatment with DBI were not found to deviate substantially from control levels. Serum PBI concentrations averaged 6.91 μg. per cent during the control period and 6.69 μg. per cent after five to twenty days of DBI therapy. Random studies done after six to twelve months of treatment indicate no effect of DBI on protein bound iodine levels. Iodine^{131} uptake studies before and after five to twenty days of treatment also indicated that DBI does not influence this function. A mean increase in uptake from 16 to 23 per cent was recorded, but in no instance was uptake elevated beyond the upper limit of normal (37 per cent); and some individuals displayed a drop in uptake. No major changes in serum cholesterol were recorded; and nearly all values were found to be within normal ranges both before and after treatment.

Adrenal function. Data obtained by measurement of twenty-four-hour urinary excretion of 17-hydroxycorticoids and 17-ketosteroids suggest that DBI administration exercised no appreciable effect on adrenal function. Values for the former averaged 19.5 mg. during the control period, and as a mean fell to 17.7 mg. during therapy while average levels of the latter fell from a mean of 10.8 to 10.2 mg. per day. Although variations of steroid excretion typical of the uncontrolled diabetic were seen, no trend suggestive of an effect of DBI on adrenal function could be established.

Liver function. Short-term estimations of the effect of DBI upon various tests reflecting liver function showed no change when values were compared with control

TABLE 1

Comparison of characteristics of diabetic patients responding and not responding to DBI

Factor	Responsive group (14)	Nonresponsive group (15)
Age (years)*	45.9 (22-68)	46.4 (10-62)
Per cent women	57	54
Duration of diabetes* (years)	1.7 (1-4)	8.6 (1-25)
Usual daily insulin dose* (units)	10.2 (0-26)	25.3 (0-50)
Per cent of group calculated to be more than 10 per cent overweight	57	7
Per cent of group in whom a prior history of ketosis was obtained	0	67
Daily dose of DBI at the time* of response or discontinuation (mg.)	195 (100-300)	196 (150-300)
Number of days of DBI therapy until response or discontinuation*	4.1 (3-6)	6.2 (3-13)
Per cent of group developing significant gastrointestinal symptoms	36	100

*Figures given as mean and range.

TABLE 2

Short-term studies of endocrine and vital organ function in twenty-nine DBI-treated patients

	Test	Before* DBI	After DBI
Thyroid function	Serum PBI (μg. per cent)	6.91 (4.8-8.8)	6.69 (5.2-7.5)
	Twenty-four-hr. Thyroidal I^{131} uptake per cent	16 (13-22)	23 (8-30)
	Cholesterol (mg. per cent)	191 (135-260)	172 (113-220)
Adrenal function	Twenty-four-hr. 17OHCS Excretion (mg.)	19.5 (7-29)	18.7 (11-27)
	Twenty-four hr. 17KS Excretion (mg.)	10.8 (5-17)	10.2 (6-16)
Liver function	Cephalin flocculation	0-1 plus	0-1 plus
	Bromsulphalein retention per cent	1.7% (1-4)	1.6% (0-4)
	Alkaline phosphatase (Bodansky units)	5.5 (4-8)	5.1 (4-7)
	Prothrombin concentration per cent	75 (68-100)	79 (65-100)
	S.G.O.T. (units)	34 (25-58)	34 (10-47)
Peripheral blood	Venous hematocrit per cent	45 (41-52)	42 (37-49)
	WBC Hundreds/mm.^{3}	9.2 (5.4-11.5)	9.5 (4.7-15.1)

*Values given as mean and range.

levels. Observations of these same indices over a six- to twelve-month period were the same. Bromsulfalein excretion values averaged 1.7 per cent after forty minutes during the control period and 1.6 per cent during treatment. Alkaline phosphatase, cephalin flocculation tests, one-stage prothrombin times, and levels of serum oxaloacetic transaminase also did not deviate from normal.

Peripheral blood. Frequent measurements of the percentage of packed red cells and total and differential white blood cell counts were made during the hospital period in all patients, and after prolonged DBI treatment in the responsive group. These data suggest that the drug does not influence these indices of hematopoietic function.

SUMMARY AND CONCLUSIONS

DBI was given to twenty-nine patients with uncontrolled diabetes mellitus. Fourteen demonstrated an adequate hypoglycemic response and became aglycosuric. This group was characterized by having mild diabetes of short duration, frequently associated with obesity. A patient with a total pancreatectomy, two juvenile diabetics, and patients who gave a history of ketosis did not respond. Reactions to DBI, other than occasionally encountered generalized weakness, were entirely gastrointestinal and included epigastric discomfort, metallic taste, nausea, vomiting, and frequent defecation. All reactions were transient and regressed promptly with cessation of therapy.

DBI in the doses given did not influence thyroid or adrenal function as measured by the usual indices. No evidence of untoward effects upon renal, hepatic, or hematopoietic function was observed during a period of one year's study.

SUMMARIO IN INTERLINGUA

Studios Metabolic E Endocrin Con Phenethyldiguanido

Phenethyldiguanido (DBI) esseva administrate a vintinove patientes con non-coercite diabete mellite. Decequatro monstrava un adequate responsa hypoglycemic e deveniva aglycosuric. Iste gruppo se distingueva per le facto que in illo le diabete esseva leve e de breve duration, frequentemente associate con obesitate. Un patiente previemente subjicite a pancreatectomia total, duo patientes con diabete juvenil, e un patiente con un historia de cetosis non respondeva. Le reactiones lateral provocate per DBI—a parte le occurrentia de debilitate generalisate in certe casos—esseva exclusivemente gastrointestinal. Illos includeva disconforto epigastric, gusto metallic, nausea, vomito, e frequentia de defecation. Omne le reactiones esseva transitori e regredeva promptemente con le interruption del therapia.

DBI, in le doses usate in le presente studio, non influentiava le functionamento thyroide o adrenal, secundo le indices in uso general. Nulle signo de effectos adverse super le functionamento renal, hepatic, o hematopoietic esseva observate in le curso del studio que habeva un duration de un anno.

ACKNOWLEDGMENT

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Diabetic Neuropathy

Review of Literature and a Case Report with Post-mortem Findings

Max Ellenberg, M.D., and Leo Krainer, M.D., New York

Diabetic neuropathy is receiving increasing recognition clinically, and the diagnosis is being made more readily although with reservation.[1] Nevertheless, the etiology, pathology and treatment remain obscure. The nature of the pathologic changes would appear to offer a direct approach obtainable without undue difficulties. And yet the paucity of documented pathologic studies in the literature is striking.

REVIEW OF LITERATURE

In 1929, Woltman and Wilder,[2] in their classic paper, reviewed the literature on this subject. They properly emphasized that all prior reports were presented before the introduction of insulin and serologic testing; the histologic technics were much less advanced; many reports were abbreviated and lacking in essential detail; and in addition many cases were complicated by cachexia, tuberculosis, etc., which could well have affected the clinical and pathologic findings. All of these objections leave little of basic referable material. Of the forty-two cases they reviewed, twenty-four had involvement of varying degrees in various parts of the spinal cord; in sixteen there was predominating involvement of the posterior columns; in four the posterior roots; and in eight, lesions in the anterior horn predominated. In twenty-four cases there was degeneration in peripheral nerves.

Both the cord and peripheral nerves were not studied in all cases. Pryce[3] reported the cases of three diabetic patients with ataxia and gangrene who died in coma; two of these had no study of the spinal cord but all showed peripheral nerve pathology. No attempt was made by this author to evaluate the element of cachexia developing in the pre-insulin era, and there is no way of eliminating the possible presence of lues. Similarly, Williamson[4] reported three cases of diabetic coma associated with florid tuberculosis. All three patients showed posterior column involvement of the spinal cord but no peripheral nerve studies were done.

Included in the report of Woltman and Wilder were ten of their own cases. In six of these, observations were confined to the peripheral nerves in amputated extremities. The amputation was always secondary to gangrene resulting from severe peripheral vascular disease. It is not surprising, therefore, that all of the specimens showed marked arteriosclerosis. Of the remaining four cases, one had no cord examination and another had no clinical evidence of a neuropathy.

Wilder[5] himself since has stated that he no longer feels that neuropathy in diabetes is necessarily arteriosclerotic in origin, contrary to the original conclusion.

A review of reported autopsy and biopsy findings from 1929 to the present includes these studies, chronologically listed:

In 1930, Root and Rogers[6] reported a diabetic patient with lower extremity paralysis. Post-mortem examination of the spinal cord revealed slight, diffuse gliosis of the dorsal column in the lumbar and thoracic areas.

Gill,[7] 1936, reported a case of neurogenic vesical dysfunction. Autopsy revealed evidence of degeneration of the cord and nerve roots in the thoracic, lumbar, and sacral regions, especially involving the posterior roots and dorsal columns of Gall and Burdach; there was also some rarefaction and atrophy of the nerve cells in the anterior horn. Only mild arteriosclerosis was noted.

Griggs and Olsen,[8] 1937, observed a case that presented with the clinical syndrome of dorsolateral spinal cord degeneration. Autopsy revealed clear-cut degeneration in the posterior funiculus, especially in the cervical and thoracic regions—"there were degenerative lesions of the posterior and lateral funiculus of the spinal cord chiefly in the cervical area. The posterior roots showed mild changes and only in those segments in which the lesion extended into the posterior horn."

Alderman,[9] 1938, presented a brief report of a post-mortem examination of a diabetic patient who had flaccid paralysis of the lower extremities. The spinal cord was shrunken and rubbery. Microscopically, the anterior horn cells were severely damaged or replaced by fat. The anterior nerve roots and peripheral nerves of the cauda equina displayed marked degenerative changes. There was a moderate amount of arteriosclerosis. In this instance, the pathologic involvement indicated an almost selective attack on the anterior horn cells.

Foster and Basset,[10] 1947, reported the pathologic findings in a case of diabetic neurogenic arthropathy which led to amputation. The peripheral nerves in the amputated specimen showed severe degenerative changes. However, they believed, on the basis of the clinical picture, that the lesion must have stemmed from the posterior roots and posterior root ganglia, although these structures were not examined.

Muri,[11] 1949, also reported a case of neurogenic arthropathy in a diabetic patient. Post-mortem examination showed a few myelin sheaths to be degenerated in the posterior tibial nerve. Sections of the lumbar cord showed some swelling of the cells of the anterior and lateral horns. The roots were normal and there was no definite disappearance of neurons. He felt that the microscopic findings in the nervous system did not adequately explain the clinical features.

Zucker and Morder,[12] 1952, described the only recorded instance of a Charcot spine as a manifestation of diabetic neuropathy. Post-mortem examination revealed extensive degeneration of the myelin sheaths of the tibial nerve and some degeneration of the myelin sheaths of the lumbar posterior roots with patchy degeneration in the fasciculus gracilis of the lumbar and thoracic cords.

Martin,[13] 1953, while conducting a study of autonomic nerve

From the Department of Medicine and the Department of Pathology, Division of Neuropathology, The Mount Sinai Hospital, New York, New York.

involvement in the lower extremities in diabetic neuropathy, obtained biopsy specimens in ten cases. All ten reportedly showed degenerative changes consisting of reduction in axis cylinders and in myelin sheaths. The cord was not studied.

Fagerberg,[14] 1956, performed routine sural nerve biopsies on patients with diabetic neuropathy and apparently found demyelinization as the basic pathologic change. He attempted to correlate these findings with the frequency of simultaneous occurrence of retinopathy and nephropathy. It is his contention that all three lesions result from involvement of the smaller blood vessels.

Dreyfus, Hakim and Adonis,[15] 1957, recorded the only pathologic study of the oculomotor nerve in a diabetic with paralysis of this nerve. The findings consisted essentially of a fusiform enlargement of the retro-orbital part of the nerve with destruction of some of the myelin sheaths and axis cylinders: evidence of regeneration in the area of destruction; Wallerian degeneration in the distal segment; and axonal reaction in the left third nucleus in the midbrain. Although the authors thought the lesion compatible with an "incomplete" ischemic neuropathy, serial sections failed to reveal an occluded artery or vein.

In 1958, Duncan, MacFarlane and Robson[16] reported a case of "pancreatic" diabetes in a patient who had retinopathy, nephropathy and neuropathy. The last was manifested by nocturnal diarrhea with incontinence, a neurogenic bladder with incontinence, and neuropathy of the lower extremities. Autopsy findings in the nervous system consisted of complete demyelinization and fibrous replacement of the peripheral nerves, and demyelinization and fibrosis of posterior nerve roots. No abnormalities were seen in the spinal cord or brain.

It is evident from the above review that there is conflicting evidence as to the pathogenesis of the observed changes, localization of histologic abnormalities, and the relative intensity of involvement. Because of these uncertainties and meagerness of available information, the following case is presented. This case is considered to be of interest and importance because the patient was followed over a period of sixteen years; neuropathy occurred while under supervision; the course of the neuropathy was followed until the time of his death; and an autopsy was performed.

CASE REPORT*

The patient, a sixty-five-year-old white male, was under observation for sixteen years. There were frequent routine clinical examinations because of a known heart murmur associated with bouts of cardiac arrhythmias. In the six years prior to his last hospitalization he had two episodes of myocardial infarction and several episodes of acute coronary insufficiency precipitated by paroxysmal tachycardia. Repeated urinalyses and several fasting blood sugars were normal.

Six months before his fatal myocardial infarction he complained of pain in the low back region. This subsided but was followed in ten days by pain in the right lower extremity associated with stiffness and a pulling sensation in the right thigh. The pain was worse at night, seriously interfering with his sleep. There were no sphincter disturbances.

On examination the blood pressure was 180/98 mm.Hg; the heart was enlarged to the left with systolic murmurs at the apex and over the aortic base; cranial nerves were intact; in the fundi there was moderate sclerosis without hemorrhages or exudates; there was marked diminution of the right knee jerk as compared with the left; right Babinski sign was present; vibration sense was absent below both iliac crests; there was slight but distinct weakness of dorsiflexion of the right foot, knee and hip; the peripheral pulses were palpable.

Laboratory examination revealed the following: blood count, blood Wassermann and extensive blood chemical determinations were normal except for a fasting blood sugar of 121 mg. per 100 ml.; urinalysis was negative except for 1-plus albuminuria. Roentgenogram of the chest showed the heart enlarged to the left with the aorta tortuous and elongated; X-ray study of the lumbar spine, right femur and hip showed no significant abnormality. Lumbar puncture yielded clear, colorless fluid under normal pressure, with normal manometric readings, cell count, Wassermann and colloidal gold. The total spinal fluid protein was elevated to 84 mg. per 100 ml. A glucose tolerance test showed blood sugar readings of 129 mg. per 100 ml. fasting; 246 mg. per 100 ml. after one-half hour; 316 mg. per 100 ml. after one hour; 281 mg. per 100 ml. after two hours; 183 mg. per 100 ml. after three hours; the last three urine specimens contained 4-plus sugar.

The onset, findings, spinal fluid protein elevation, the abnormal glucose tolerance test, the family history, and the course, all indicated the presence of diabetes and diabetic neuropathy.

The further course of this patient is of interest in that within three weeks after onset, improvement in power began. This progressed so that, within two months, function was almost completely normal. However, although the Babinski sign disappeared, the right knee jerk persisted as slightly hypoactive and the tonus in the right quadriceps muscle remained decreased. Six months after onset he sustained acute coronary occlusion and died.

AUTOPSY FINDINGS

Macroscopic diagnoses: Recent occlusion of the left anterior descending coronary artery due to arteriosclerosis. Rheumatic aortic and mitral stenosis. Cardiomegaly. Pulmonary edema. Dilatation of the proximal part of the main pancreatic duct.

Microscopic diagnoses: Focal interstitial fibrosis of the pancreas. Minimal arterio- and arteriolosclerosis of kidneys without evidence of Kimmelstiel-Wilson lesions. Moderate arteriosclerosis of both coronary arteries with hemorrhage beneath an arteriosclerotic patch in the left anterior descending branch.

Neuropathologic findings: Permission for a partial postmortem examination limited the studies of the nervous system to the lower part of the spinal cord and cauda equina (total length 12 cm.), the right and left sciatic nerves in their retroperitoneal course (12 cm. and 10 cm., respectively) and one unnamed nerve, presumably the femoral.

Spinal cord: A focus of incomplete malacia was seen in L_2 and L_3 in the right posterior tract (figure 1). Within this focus the intraspinal vessels showed some arteriolosclerosis. Slight pallor of the ventral posterior tract was noted in L_5 and S_1, and there was slight pallor of the lateral pyramidal tracts in L_5.

Spinal roots: There was patchy fibrosis, with occasional regenerating bundles indicated by rows of oblong nuclei in both the anterior and posterior roots.

* This case was previously reported in greater detail, as an illustration of diabetic neuropathy presenting as the initial clinical manifestation of diabetes.[17]

FIG. 1. Spinal cord, upper lumbar segment. Focal myelomalacia in the right posterior tract.

Nerve root: (the portion distal to the spinal ganglion before complete intermingling of the anterior root fibers and preganglionic sensory fibers takes place). In the right fifth lumbar root nerve there was marked fibrosis. This was a result of severe thickening of the endoneurium affecting the whole diameter of the nerve, but some myelinated fibers were still recognizable in this area (figure 2).

Right sciatic nerve: Some bundles contained minimal thickening of the endoneurium while others showed patchy fibrosis (figure 3). In one bundle, the normal pattern was completely replaced by proliferated fibrous and cellular bands in which occasional degenerating and numerous delicate regenerating myelinated fibers were enclosed (figure 4). Branching axis cylinders were seen in this area. In the bundle so involved, arteriolosclerosis of endoneurial vessels was noted. Gross section distal from the above showed patchy endoneurial thickening in some bundles and areas of hyaline scarring. In the hyaline areas axis cylinders were absent.

Left sciatic nerve: Diffuse, patchy endoneurial fibrosis and also normal bundles were seen. The process, although of the same nature, was much milder than in the right sciatic nerve.

Unnamed nerve (femoral?): Only occasional minimal endoneurial fibrosis was noted.

DISCUSSION

The problems that one might attempt to clarify on the basis of pathologic studies include: (1) the site of lesions; (2) the pathogenesis; and (3) clinical-pathologic correlation.

The neuropathologic lesions in diabetic neuropathy, as judged from the available reports in the literature, may involve many different parts of the spinal cord, roots, and peripheral nerves, singly and in combination. In our case, pathologic alterations were present in all three areas. However, the nature of the changes indicates that, whereas those in the peripheral nerves represented a chronic pre-existing lesion, the lesions in the root and cord were relatively recent. Hence the root and cord were the sites of the lesions responsible for the presenting picture.

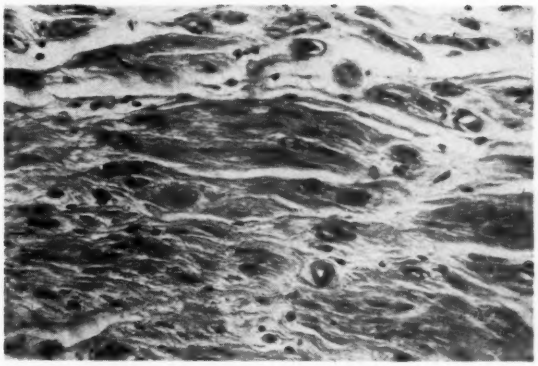

FIG. 2. Nerve root showing marked fibrosis.

The pathogenesis of the lesion in diabetic neuropathy is shrouded in considerable doubt and disagreement. Vascular lesions with consequent interruption of blood supply to the involved nerves have repeatedly been implicated. Some of the reasons for this are: (1) the increased incidence of neuropathy in the age group over forty; (2) the frequent association of vascular disease and diabetic neuropathy; and (3) the generally high over-all frequency of vascular disease and peripheral gangrene in diabetes. Fagerberg[18] has attempted to explain all the neurologic findings on the basis of involvement of the small vessels of peripheral nerves, comparable to the anatomic changes in retinopathy and nephropathy. Nevertheless, the autopsies referred to previously in this paper place very little stress on the presence or significance of blood vessel involvement.

There are several clinical features that make vascular involvement as the basis of diabetic neuropathy untenable in many instances. These include: (1) the relative rarity of neuropathy in nondiabetics who have evidence of severe and advanced obliterative arteriosclerosis; (2) the acute onset; (3) the pronounced improvement which so many of these patients experience; (4) frequent involvement of the autonomic nervous system; and (5) its occurrence in patients with no evidence of any vascular involvement in either large or small vessels. It should be stressed that if these lesions are vascular in origin, it is most likely the smaller blood vessels, i.e., the arterioles, that are involved. Thus the absence of arteriosclerosis of the vasa nervorum which frequently has been emphasized need not be significant.

As to the other possible causative agents, no toxins or significant changes in the intermediary metabolites have been consistently demonstrated. The possibility of vitamin B deficiency has been entertained only to be discarded. Root[19] felt that although there is no doubt that

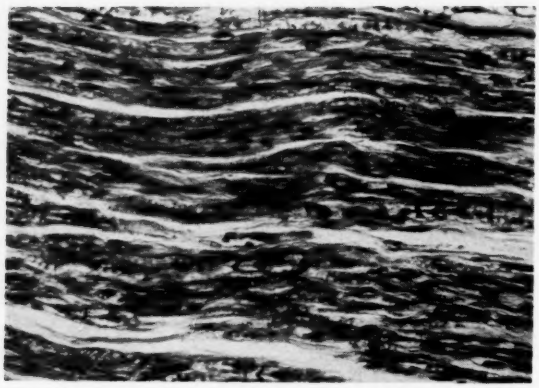

FIG. 3. Right sciatic nerve showing patchy endoneurial fibrosis.

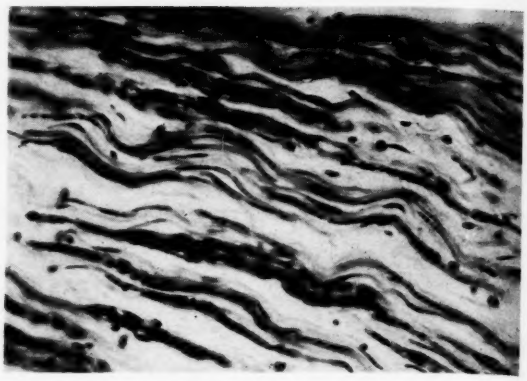

FIG. 4. Area in right sciatic nerve containing delicate regenerated myelinated fibers within the fibrous bands.

age and vascular disease make fertile soil, diabetes itself produces directly or indirectly some specific effect.

The pathogenesis of the lesions in our case is not readily apparent. The possibility of a vascular origin exists in view of the tendency for the most severe lesion to correspond generally to the areas of most evident arteriolosclerosis; but no occlusion of vascular channels was demonstrated. The situation in the peripheral nerves differs in that the lesion appeared to antedate the clinical picture. Here, the chief characteristic is the varying pattern of severe lesions with relatively well preserved neighboring fibers in the same nerve bundles. This suggests a vascular etiology and a similar pattern may be seen in the neuropathies found in periarteritis nodosa. However, the evidences of regeneration in areas of previous degeneration is contradictory to this interpretation unless one postulates the development of collateral channels of circulation; these were not in evidence. Similar regenerative activity was present in the affected oculomotor nerve as described by Dreyfus et al.[15] and in the case of Foster and Bassett.[10]

The clinical impression of the probable underlying pathology presents another area of differences. Many clinicians, basing their views on clinical observation, neurologic examination, and spinal fluid findings, have felt that the pathologic change must be located centrally, i.e., in the spinal cord and/or nerve roots (Kraus,[20] Root and Rogers,[6] and Joslin and Root).[21] De Jong[22] stated that although many findings of diabetic neuropathy could be explained on the basis of peripheral nerve involvement, there were some features, including changes in proprioceptive sensation, ataxia, and pupillary changes, which could only be explained on the basis of central pathology. On the other hand, Goodman and his associates[23] are representative of a group that believe that all manifestations of diabetic neuropathy may be explained on the basis of peripheral nerve alteration.

In our case, certain aspects of the clinical picture, namely the Babinski sign, elevation of the spinal fluid protein, and the distribution of muscular weakness all indicated pathology in the cord and root. It was interesting, therefore, to note that there were lesions in the cord and root, thus offering at least a partial clinical-pathologic correlation. The extent of alteration may have been modified by the lapse of six months from the time of onset to his death; it should be pointed out, also, that he had improved clinically.

It is difficult to determine the clinical significance of the pathologic alterations in the peripheral nerves. The acute onset and relatively rapid regression, which were readily compatible with the root and cord findings, could not be explained on the basis of the peripheral nerve findings. This statement is based on the presence of marked regeneration which was incompatible with the known slow rate of nerve recuperation and thus indicated a long-standing process. The histologic picture suggested a chronic process with a relative balance of degeneration and regeneration, so that under ordinary circumstances there would be no significant symptomatology. The superimposition of the root and cord lesions would thus not only produce their own symptomatology but would also precipitate the latent peripheral nerve pathology into the clinical picture.

It is apparent that many more pathologic studies, including biopsies and autopsies with clinical correlation in diabetics with and without nerve involvement, must be obtained. The presence of more than one type or site of lesion would not be at all surprising. This statement derives from the fact that diabetic neuropathy is most protean in its manifestations. The symptoms may in-

dicate involvement of the peripheral nerves, spinal roots, cord, cerebrum or any combination thereof. Although the most frequent clinical picture is characterized by involvement of the nerves of the lower extremities, usually bilaterally symmetrical and predominantly sensory, unilateral involvement may be present; there may or may not be pain; there may be severe pain, with a minimum of objective findings; there may be absence of pain, with a multiplicity of findings; there may be complete peripheral sensory loss without paralysis, or there may be grave paralysis with insignificant sensory involvement; there may or may not be associated vascular changes; the diabetes may be mild or severe. There may be cerebral and cranial nerve involvement, as indicated by ocular-nerve palsies; pupillary abnormalities; trigeminal and facial nerve involvement, and occasionally emotional disturbances severe enough to lead to psychiatric diagnoses. There may be autonomic nerve and visceral involvement, including changes in vasomotor, pilomotor, sudomotor control; dependent edema; abnormality of texture and consistency of the skin; neuropathic ulcers; Charcot arthropathy; impotence; orthostatic hypotension; neurogenic vesical dysfunction; and neurogenic bowel dysfunction. There may be a syndrome referred to as "pseudotabes diabetica," characterized by diminution or loss of deep reflexes, sensory changes, lightning pains, ataxia of the sensory type, bladder and bowel involvement, and pupillary abnormalities.[1]

In view of the complexity and variability of the observed clinical pictures, differing sites and causes of pathologic changes may be expected. However, the necessity for grouping of these cases through clinical pathologic correlation is a challenge that has not been satisfactorily met and calls for intensive attack.

SUMMARY

1. The neuropathologic findings in a patient with diabetic neuropathy are presented and discussed.

2. The more recent lesions in this case were in the root and dorsal cord.

3. The peripheral nerve lesions appeared chronic; their contribution to the presenting clinical picture was probably less important than that of the lesions in the nerve roots and dorsal cord.

4. The necessity for more intensive pathologic study with clinical correlation in diabetic neuropathy is stressed.

SUMMARIO IN INTERLINGUA

Neuropathia Diabetic

1. Es presentate e discutite le constatationes neuropathologic in un patiente con neuropathia diabetic.

2. Le plus recente lesiones in iste caso esseva in le radices e le cordon dorsal.

3. Le lesiones de nervo peripheric pareva esser chronic. Lor contribution al tableau clinic de presentation esseva probabilemente minus importante que illo del lesiones in le radices nerval e le cordon dorsal.

4. Es sublineate le necessitate de plus intense studios pathologic con correlationes clinic in le campo del neuropathia diabetic.

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Effect of Prolonged Administration of Tolbutamide in Depancreatized Dogs

Anna Sirek, M.D., Otakar V. Sirek, M.D., Ph.D., Y. Hanus, F. C. Monkhouse, Ph.D., and C. H. Best, M.D., Toronto

In a preliminary communication[1] we have reported that depancreatized dogs treated with tolbutamide for prolonged periods showed derangements of liver function. The present paper gives a detailed account of our studies on twelve depancreatized dogs treated orally with various doses of the drug. When given relatively large doses, the animals required appreciably smaller amounts of exogenous insulin to maintain a standard degree of control.

MATERIALS AND METHODS

The series represents a total of sixteen dogs and consists of the following groups of animals:

1. Depancreatized adult dogs

(a) Two dogs maintained on 150 mg. per kilogram body weight of tolbutamide; (b) three dogs on 100 mg. per kilogram body weight; (c) three dogs on 30 mg. per kilogram body weight; (d) two controls.

2. Depancreatized pups

(a) Three pups maintained on 70 mg. per kilogram body weight; (b) one control.

3. Partially depancreatized adult dogs (approximately 50 per cent of pancreatic tissue removed from the splenic end):

(a) One dog maintained on 30 mg. per kilogram body weight; (b) one control.

The adult animals weighed about 10 kg. and the pups, which at the time of pancreatectomy were approximately six months old, about 5 kg. The diabetic dogs were fed measured amounts of a commercial ration supplemented with raw pancreas. They were kept in metabolism cages and the urine was collected daily. The total amount of sugar was determined by Clinitest* tablets. Regular and Protamine Zinc insulin were administered daily, prior to feeding, in two separate subcutaneous injections in amounts required to keep blood sugar levels on the average below 200 mg. per 100 cc. and the glycosuria very slight. Because of considerable fluctuation from day to day and week to week, it was found more convenient to express the effect of tolbutamide upon blood sugar in terms of the amount of insulin spared. The diabetic dogs were kept under these standard conditions for at least two weeks and in some cases much longer before treatment was commenced.

Blood samples were obtained frequently before and during the test period. Blood sugar was determined by the micro-micromethod described by King.[2] Bromsulphalein clearance tests were performed according to Gornall and Bardawill.[3] Serum bilirubin was determined according to Malloy and Evelyn.[4] Prothrombin clotting time was determined in whole plasma and in 25 and 50 per cent saline dilutions by the one-stage method of Quick.[5] Total cholesterol was determined by a modification of the method of Bloor, Pelkan and Allen.[6] Total serum protein was determined by the method of Weichselbaum.[7] Serum albumin was determined by salt precipitation with 26 per cent sodium sulphate[8,9] and the precipitated globulins were separated by centrifugation under ether according to Kingsley.[10] Serum alkaline phosphatase was estimated by the amount of phenol liberated from a phenolphosphate substrate and expressed in King-Armstrong units per 100 ml. of serum.[11] Serum glutamic-pyruvic and glutamic-oxalacetic transaminases were determined by a procedure in which the oxalacetate and pyruvate formed an intensively brown-colored hydrazone; the serum levels were expressed in Sigma-Frankel units per 1 ml. of serum.[12] Total serum protein-bound hexose was determined with the orcinol reagent according to Lustig and Langer[13] using Winzler's modification.[14]

RESULTS

The first change in liver function noted at any dose level was usually an elevation of the serum alkaline phosphatase. Figures 1-a,b,c show the behavior of the alkaline phosphatase in the first two groups of animals. The same elevation was observed in the partially depancreatized dog. A fall in total serum proteins was also encountered which was due to a decrease in serum albumin. Figures 2-a,b,c present the results obtained in the same groups of animals. Again, the partially depancreatized animal showed the same change. The glutamic-oxalacetic and glutamic-pyruvic transaminase levels became elevated and the highest values as well as the normal ones are recorded in table 1. The serum cholesterol levels were not followed closely, but in several dogs a profound fall was encountered in the terminal stages. The lowest figures as well as the control values for each animal are included in table 1. Total protein-bound hexose was determined on a number of blood samples and an elevation from the average normal of 106 mg. per 100 cc. up to 153 mg. per 100 cc. was found in all our dogs treated with tolbutamide.

From the Banting and Best Department of Medical Research and the Department of Physiology, University of Toronto, Toronto, Canada.

* Ames Company, Inc.

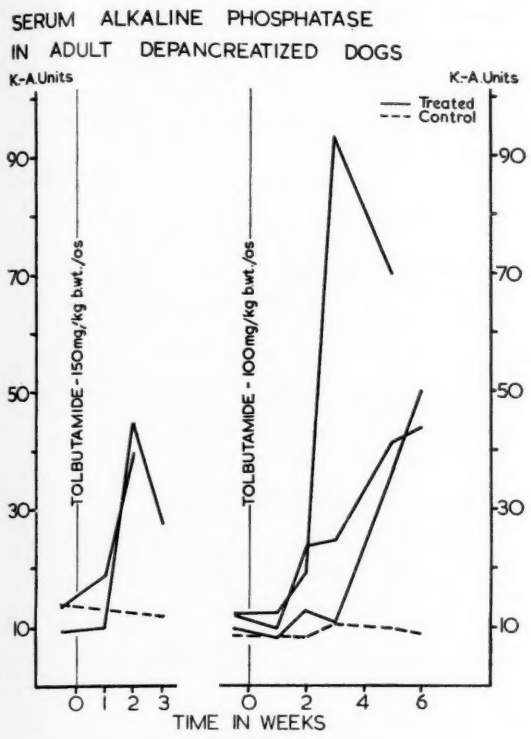

FIGURE 1A

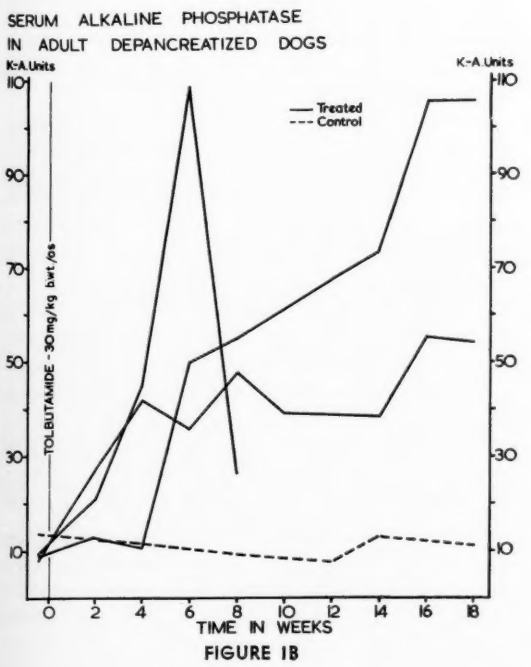

FIGURE 1B

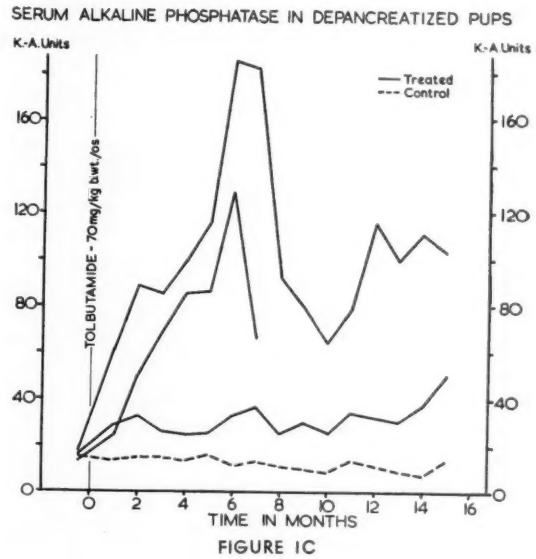

FIGURE 1C

TABLE 1

Serum transaminase and cholesterol values before and during tolbutamide treatment

Dose/kg. b.wt.	TGO—units control	TGO—units highest	TGP—units control	TGP—units highest	Cholesterol mg. per cent control	Cholesterol mg. per cent lowest
150	31	1,010	20	230	306.2	65.9
	21	660	21	200	400.5	67.6
100	—	25	—	28	—	229.6
	—	14	—	18	—	156.6
	—	380	—	200	—	—
70	16	71	18	72	194.0	156.6
	14	78	24	240	288.4	131.0
	11	98	11	120	201.1	34.6
30	17	194	19	800	314.0	120.0
	23	155	21	670	251.0	90.8
	15	130	3	120	261.7	184.2
	* —	1,102	—	960	—	27.3

* Partially depancreatized.

Bromsulphalein clearance tests were performed on all animals frequently and the results were found to be within normal limits, i.e., less than 10 per cent retention fifteen minutes after injecting 10 mg. per kilogram body weight.

The insulin requirements are recorded in figure 3. It can be seen that those dogs which were maintained on 100 mg. or 150 mg. per kilogram body weight of tolbutamide required, after a certain time, considerably less insulin. The insulin requirements of the puppies which have been maintained on 70 mg. of tolbutamide per kilogram body weight for almost sixteen months, (and thus are now adult dogs) and the insulin requirements of the dogs maintained on 30 mg. per kilogram body weight have not appreciably changed. These figures

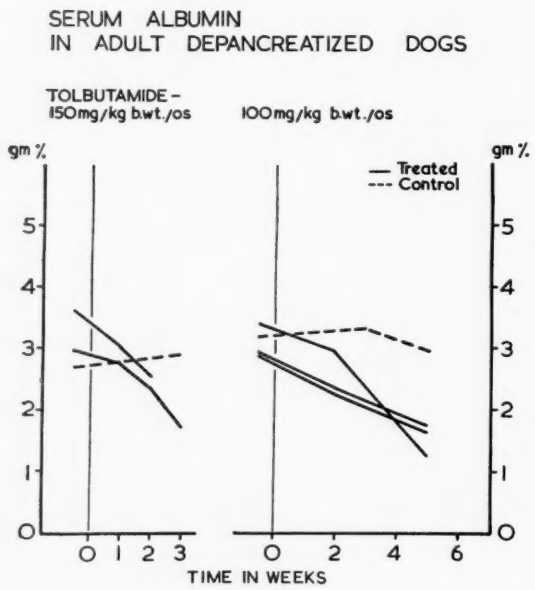

FIGURE 2A

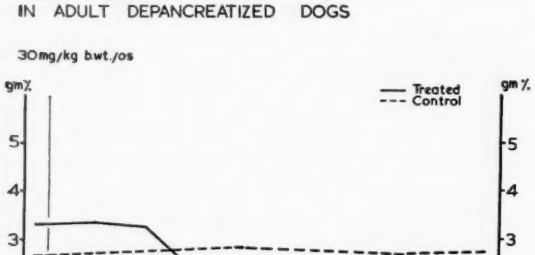

FIGURE 2B

are omitted from the graph. The partially depancreatized dog did not require exogenous insulin at any time and showed a moderate but substantial fall in blood sugar directly after the first dose of tolbutamide. In the totally depancreatized dogs which responded to the drug with a fall in blood sugar, there was no immediate drop after the first dose, but the blood sugars gradually fluctuated at lower levels and thus enabled us to decrease the daily amount of injected insulin.

The prothrombin clotting time in plasma diluted with saline to 25 per cent was in some cases prolonged to 300 seconds, in comparison with about twenty seconds

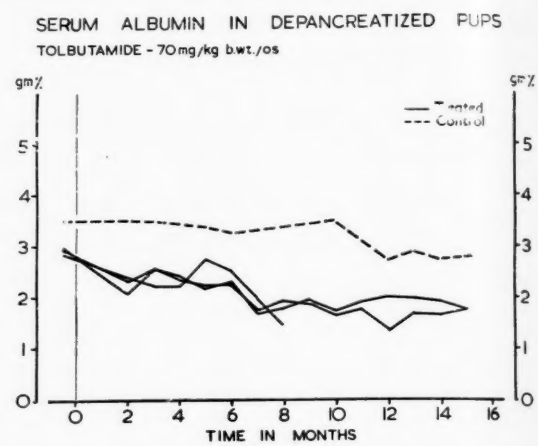

FIGURE 2C

for normal dogs. This prolongation usually could be eliminated by parenteral administration of vitamin K_1. In two animals the prothrombin clotting times were elevated in the terminal stages and here vitamin K_1 was without effect. In two other animals bleeding into various organs and into the peritoneal cavity was encountered but the prothrombin clotting time was normal. The dying animals were always jaundiced. The elevation in serum bilirubin levels was due to a rise in direct reacting bilirubin. It appears then that bleeding tendencies, prolonged prothrombin clotting times and jaundice were abnormalities which occurred independently of each other but a final conclusion on this point is not possible in this small series of animals.

All five dogs treated with 100 or 150 mg. tolbutamide per kilogram body weight deteriorated within three to six weeks and either died or had to be sacri-

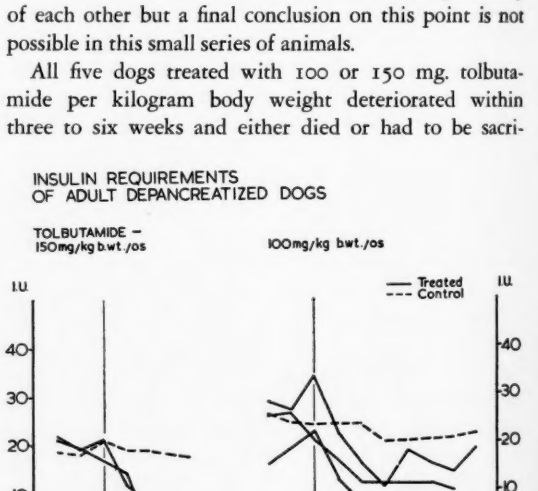

FIGURE 3

ficed. One animal in each of the other groups, i.e., those receiving less than 100 mg. tolbutamide per kilogram body weight, followed the same course as those receiving the higher doses. The gross and microscopic findings in all of our autopsied animals will be reported in a separate communication. The completeness of the pancreatectomy was verified in each case.

DISCUSSION

The data presented above indicate that the livers of our experimental animals have been seriously affected by tolbutamide. The data also show that prolonged administration of the drug in doses of 100 mg. and 150 mg. per kilogram body weight may produce a fall in blood sugar in depancreatized dogs and diminish but not abolish their requirements for exogenous insulin. A fall in blood sugar after prolonged administration of tolbutamide to depancreatized dogs was observed by Ricketts and associates[15] and Schambye.[16] Bleeding into organs of depancreatized dogs treated with large doses of tolbutamide was reported previously by Schambye,[17] but he did not conduct any extensive study of liver function. In another study Tarding and Schambye,[18] using C^{14}-labeled glucose, were able to demonstrate a reduction in hepatic glucose output after a single injection of tolbutamide to normal dogs. Ashmore, Cahill, Earle and Zottu,[19] by cannulating the portal and hepatic veins, provided direct evidence of a diminished glucose output from the liver when normal dogs were given a single intravenous injection of tolbutamide. These results would suggest that an analogous situation may have existed in our depancreatized animals receiving 100 mg. or more per kilogram body weight of the drug for prolonged periods. The diminished insulin requirements of our animals may prove to be due to a diminished glucose output from the liver. It would also be indicated that doses such as 30 mg. or 70 mg. per kilogram body weight, while effective in producing the described changes in liver function, were perhaps insufficient to interfere with glucose output as judged by the relatively steady blood sugar levels and insulin requirements. This suggestion is strengthened by the numerous reports on experiments with liver slices. Tolbutamide had to be present in the incubation medium in rather large concentration in order to depress the activity of enzymes involved in the release of free glucose from this organ.[20–24]

It might be argued that the derangements in liver function observed in our dogs are the result of nutritional deficiencies due to malabsorption. All our depancreatized animals were kept under standard conditions for from several weeks to one year before they were given tolbutamide. None of the dogs showed any evidence of digestive or hepatic disturbance during the control period and these observations and the results of our previous experiences with depancreatized dogs on similar diets and insulin therapy tend to rule out nutritional deficiencies as a cause of the hepatic changes. In the partially depancreatized dog the pancreatic tissue was unable to protect the animal from the toxic effects of the drug. In recent experiments we have observed that even normal dogs, when treated with small amounts of tolbutamide, may show derangements in liver function similar to those described in the depancreatized animals.

The severe change in liver function found in dogs is in a striking contrast to the results of similar studies in human subjects. We have carried out the same liver function tests in a series of human diabetics treated with tolbutamide by our clinical colleague, Dr. B. Leibel, and in accord with other authors[25] have thus far seen no abnormalities. The results of the study of Mohnike and Wittenhagen[26] indicate that in the dog this drug is metabolized in a different way from that occurring in man. The possibility that the tolbutamide treated dog produces a toxic metabolite either in the form of para-toluol-sulfonamide or some other by-product is now being investigated.

SUMMARY

Tolbutamide (Orinase-Hoechst) was given in various doses orally to twelve depancreatized dogs for prolonged periods of time. All the animals suffered derangement in liver function, two died and six had to be sacrificed because they became jaundiced and were deteriorating rapidly. It was observed, in confirmation of other authors (Ricketts, Schambye), that depancreatized animals when treated with relatively large doses of tolbutamide may require appreciably smaller amounts of exogenous insulin to maintain a standard degree of control. The present findings are discussed in the light of those of other workers.

SUMMARIO IN INTERLINGUA

Effecto Del Administration Prolongate De Tolbutamido In Canes Dispancreatisate

Tolbutamido (Orinase-Hoechst) esseva administrate in varie doses per via oral a dece-duo dispancreatisate canes durante prolongate periodos de tempore. Omne le animales suffreva un disrangiamento del function hepatic. Duo moriva, e sex debeva esser sacrificate proque illos disveloppava jalnessa e se deteriorava rapidemente. Esseva observate, in confirmation de altere autores (Ricketts, Schambye) que dispancreatisate animales—quando tractate con relativemente grande doses de tolbutamido —require in certe casos appreciabilemente plus micre

quantitates de insulina exogene pro mantener un grado standard de controlo. Le presente constatationes es discutite in le lumine de illos de altere autores.

ACKNOWLEDGMENT

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The metabolic blocks thus far discussed have a direct relation to the production of genetic disease. The relation, however, may not always be so direct, and the pathological consequences of a gene may manifest themselves only in specific environmental situations. It has recently been shown, for example, that a genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase may cause an alteration in glutathione metabolism, resulting in an instability of reduced glutathione.[1] As a consequence of this instability, the red blood cells are liable to hemolysis following the ingestion of certain drugs such as naphthalene, primaquinine, sulfanilamide, and nitrofurantoin. Thus an induced hemolytic anemia develops, which, though appearing to be environmentally produced, has a definite genetic basis. From a practical standpoint, an assay of blood cells for this enzyme may make it possible to detect those individuals who are drug-sensitive and would be harmed by these drugs.

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Diabetes Mellitus at Ages One to Five

Findings at Onset

Howard S. Traisman, M.D., John J. Boehm, M.D., and Alvah L. Newcomb, M.D., Chicago

From 1921 through 1957, 110 infants and children who developed diabetes mellitus after their first and prior to their fifth birthday were seen at the hospitals whence this study originates. It is the purpose of this paper to record our observations of these young diabetic patients.

METHOD OF STUDY

The records of these patients obtained at the time of their first admission to the hospital were reviewed. The following major factors were investigated: sex distribution, age of onset, interval between onset of symptoms and diagnosis, symptoms or presenting complaints, infectious diseases associated with the onset of the diabetes, time of year of onset of symptoms, and hereditary factors involved.

OBSERVATIONS

Sex distribution. Of the 110 patients studied, sixty-five (59.1 per cent) were male and forty-five (40.9 per cent) female.

Age of onset of diabetes mellitus and interval between onset of symptoms and diagnosis. In this series there were none whose onset began in the first year; among thirty-two patients the onset of diabetes was between twelve and twenty-three months of age; for thirty, it was between twenty-four and thirty-five months. In twenty-one, it was discovered between thirty-six and forty-seven months of age, and in twenty-seven, between forty-eight and fifty-nine months of age (table 1).

The average interval between onset of symptoms and diagnosis in twenty-six cases between twelve and twenty-three months was two and one-half weeks with a range of seven days to eight weeks.

Among twenty-nine cases in the twenty-four- to thirty-five-month age group the average interval between onset and diagnosis was three and one-half weeks, with a range of seven days to twelve weeks.

Of the twenty cases in the thirty-six- to forty-seven-month age group the average interval was four weeks, with a range of three days to twelve weeks.

In patients between forty-eight months and five years, the interval was four and one-half weeks, with a range of four days to sixteen weeks.

The average interval for 102 cases in all age groups was three and six-tenths (table 1).

For eight patients in this series the interval between onset of symptoms and diagnosis could not be determined. The reasons for this lack of a clear onset lay in the absence of a history that could be elicited from the parents, or because the patient was admitted after the diagnosis had been established but the duration of the presenting symptoms was not precisely recorded.

Symptoms or presenting complaints. In adults with diabetes mellitus it is well known that polyuria, polydipsia and polyphagia are the most common symptoms. These presenting symptoms are also seen in juvenile diabetes but with different frequency. Table 2 lists in the order of frequency the presenting symptoms of the 110 patients in this study.

1. Polyuria—This symptom was present in 84.5 per cent of the entire series. It should be noted that the incidence is higher in the older age groups than in the twelve- to twenty-three-month age group in which it was only 72 per cent.

2. Polydipsia—Increased thirst was similarly noted in a high percentage of cases (81 per cent). This also was more frequently noted in the older children than in the twelve- to twenty-three-month age group.

3. Weight loss—This was present in 43 per cent of the patients with a slight increase in the incidence as the age of onset increased.

In 88 per cent of the patients either polyuria or polydipsia was present whereas in 91 per cent either polyuria or polydipsia, or weight loss was noted.

4. Polyphagia—Increased appetite was noted in 25 per cent of the series. There was an incidence of 44.5 per cent in the forty-eight-month to five-year group, compared to an incidence of only 12.5 per cent in the

From the Department of Pediatrics, Northwestern University Medical School, The Children's Memorial Hospital, Chicago, Illinois, and The Evanston Hospital, Evanston, Illinois. Address requests for reprints to Dr. Howard S. Traisman, 6354 North Broadway, Chicago 40, Ill.

TABLE 1

(*continued on opposite page*)

Age group (months)	Age at onset (110 patients) M	F	Total	Average interval between onset of symptoms and diagnosis No. of cases	Average interval (weeks)		Patients admitted in acidosis No.	Per cent of age group
12-23	21	11	32	26 (6)*	2.5		13	40.6
24-35	20	10	30	29 (1)*	3.5		7	23.4
36-47	13	8	21	20 (1)*	4.0		10	47.6
48-59	11	16	27	27	4.5		10	37.0
				Average	3.6	All age groups	40	36.4

twelve- to twenty-three-month age group.

5. Anorexia—Loss of appetite was found in 14.5 per cent of the total cases with a much higher frequency noted in the youngest age group (28 per cent).

6. Lethargy—Although this symptom was present in a relatively large number of cases (13 per cent), there was no striking dissimilarity of incidence among the various age groups.

7. Enuresis—Bed-wetting appearing in 6 per cent of the group.

8. Vomiting—In 4.5 per cent of the patients vomiting accompanied the onset of diabetes.

9. Less common presenting symptoms—Three children were noted to be more irritable just prior to the diagnosis of diabetes. In three patients "craving for sweets" was reported. Of three other patients the mothers noted that the diapers had a "sticky" feel to the touch, while two reported a "strong odor to the urine."

In only two children was diabetes discovered on routine urinalysis. One was a two and one-half-year-old boy whose urine was examined when he was admitted to the hospital for acute appendicitis. The other was a four-and-one-half-year-old girl whose diabetic father tested her urine when she appeared ill. In two patients the onset of diabetes was heralded by a hypoglycemic episode.

Personality change, boils, headache, and abdominal cramps—each occurred once in different subjects as a presenting complaint in the group.

Patients admitted in acidosis or coma. The term diabetic acidosis was used to describe any infant or child whose total serum CO_2 content was 15 mEq./L. or less. In addition, patients who failed to respond or who were unconscious were classified as being in coma.

Forty patients, including six in coma and comprising 36 per cent of the total group, were admitted in acidosis prior to establishing the diagnosis of diabetes. In the group twelve to twenty-three months of age there were thirteen individuals admitted in acidosis. There were seven children in the twenty-four- to thirty-five-

TABLE 2

Symptoms or presenting complaints (110 patients)

	12-23 mo. 32 patients No. and per cent of age group	24-35 mo. 30 patients No. and per cent of age group	36-47 mo. 21 patients No. and per cent of age group	48-59 mo. 27 patients No. and per cent of age group	Total 110 patients No. and per cent of total group
Polyuria	23 (71.9)	26 (86.6)	18 (85.7)	26 (96.4)	93 (84.5)
Polydipsia	19 (59.4)	27 (90.0)	18 (85.7)	25 (92.6)	89 (81.0)
Weight loss	11 (34.4)	12 (40.0)	10 (47.6)	14 (51.9)	47 (42.7)
Polyphagia	4 (12.5)	8 (26.6)	4 (19.0)	12 (44.5)	28 (25.4)
Anorexia	9 (28.1)	4 (13.3)	0 —	3 (11.1)	16 (14.5)
Lethargy	5 (15.6)	2 (6.7)	3 (14.3)	4 (14.8)	14 (12.7)
Enuresis	0 —	2 (6.7)	3 (14.3)	2 (7.4)	7 (6.4)
Vomiting	2 (6.3)	0 —	2 (9.5)	1 (3.7)	5 (4.5)
Irritability	1 (3.1)	1 (3.3)	0 —	1 (3.7)	3 (2.7)
"Craving for sweets"	0 —	0 —	1 (4.8)	2 (7.4)	3 (2.7)
"Sticky diaper"	0 —	3 (10.0)	0 —	0 —	3 (2.7)
"Strong odor to urine"	1 (3.1)	1 (3.3)	0 —	0 —	2 (1.8)
Routine urinalysis	0 —	1 (3.3)	0 —	1 (3.7)	2 (1.8)
Hypoglycemia	1 (3.1)	1 (3.3)	0 —	0 —	2 (1.8)
Personality change	0 —	1 (3.3)	0 —	0 —	1 (0.9)
Boils	0 —	0 —	0 —	1 (3.7)	1 (0.9)
Headache	1 (3.1)	0 —	0 —	0 —	1 (0.9)
Abdominal cramps	0 —	0 —	1 (4.8)	0 —	1 (0.9)

TABLE 1
(*continued from opposite page*)

Association of infections with diabetes									
Respiratory infections		Exanthems		Pyelitis		Vaginitis		Total infections for age group	
No.	Per cent age group	No.	Per cent age group	No.	Per cent age group	No.	Per cent age group	No.	Per cent
13	40.6	1	3.1	1	3.1	1	3.1	16	50.0
8	26.7	1	3.3	0		0		9	30.0
8	38.0	0		0		0		8	38.0
7	25.9	0		0		0		7	25.9
36	32.8	2	1.8	1	0.9	1	0.9	40	36.4

*For these eight cases no interval could be determined either because no onset history could be elicited from the parents or because the patient was admitted after diagnosis had been established earlier, and duration of presenting symptoms was not accurately recorded.

month age group, and ten children in the thirty-six- to forty-seven-month age group and ten in the group forty-eight to fifty-nine months old (table 1).

Six patients (5.5 per cent of the total number) were admitted in coma. Two were in the group twelve to twenty-three months old. There was one child in the twenty-four- to thirty-five-month, and one in the thirty-six- to forty-seven-month group. Two children were in the forty-eight- to fifty-nine-month age group. In the twelve- to twenty-three-month group one infant's symptoms were present one-half week prior to admission. Nothing is known of the onset of symptoms in the other infant in this group. The average duration of symptoms in the other three age groups was two weeks (table 3). This emphasizes the fact that the younger the diabetic patient the more rapid is the onset of his ensuing acidosis and coma.

Association of infections with onset of diabetes. With the onset of diabetes there were forty patients (36 per cent) in whom an associated secondary disease was found (table 1). Respiratory infections were by far the most common. These included the common cold, pharyngitis, tonsillitis, otitis media, mastoiditis, bronchitis, and pneumonitis. In 33 per cent of the entire group there was an associated respiratory infection at the time of onset with little difference in frequency among the various age groups. One patient had rubella and another rubeola associated with the onset of their diabetes mellitus. One child had pyelitis and another vaginitis.

Time of year in relation to onset of diabetes. It is not known whether or not the season of the year has any relationship to the onset of diabetes. In this series of 110 patients nineteen (17 per cent) were discovered in the spring, twenty-four patients (22 per cent) in the summer, thirty (27 per cent) in the autumn, and thirty-seven patients (34 per cent) in the winter months. Thus, 39 per cent of cases occurred in the spring and summer compared to 61 per cent in the autumn and winter (table 4). There was no apparent correlation with infection.

Family history of diabetes. For study of the incidence of diabetes in the families of the 110 juvenile diabetics, two groupings were made to include those patients with a proximate family history (diabetes in parents, grandparents, aunts, or uncles) and those with a remote family history (great-grandparents, great-aunts, great-uncles, or first cousins). Twenty-seven (25 per cent of the entire group) had a proximate family history of diabetes, and four (4 per cent) had a remote family history, making a total of thirty-one patients (28 per cent) with a family history of diabetes at the time of onset of their disease (table 5).

TABLE 3
Patients admitted in coma

Age group (months)	Number	Per cent of each age group admitted in coma
12-23	2	6.2
24-35	1	3.3
36-47	1	4.8
48-59	2	7.4
All age groups	6	5.5

TABLE 4
Time of year of onset of diabetes

Age group (months)	Spring (March-May)	Summer (June-Aug.)	Autumn (Sept.-Nov.)	Winter (Dec.-Feb.)
12-23	3	9	11	9
24-35	5	6	10	9
36-47	7	3	3	8
48-59	4	6	6	11
Total	19	24	30	37
Per cent	17.3	21.8	27.3	33.6

DISCUSSION

The predominance of male patients in the age group that we are reporting is in striking contrast to adult diabetics in whom the females outnumber the males.[1] However, it is consistent with other studies of juvenile diabetes in which the sex ratio approximates

TABLE 5

Family history of diabetes (110 patients)

Age group (months)	Proximate*	Remote†	Total
12-23	5	2	7
24-35	12	0	12
26-47	4	2	6
48-59	6	0	6
Total	27	4	31
Per cent	24.6	3.6	28.2

*Parents, grandparents, aunts, uncles.
†Great-grandparents, great-aunts, great-uncles, cousins.

unity.[2-4] We have noted an increased number of female patients in the older age groups under our care.

The diagnosis of diabetes mellitus in infants is rare. The diagnosis is seldom made because of the infrequency of the disease, its sudden onset, and the fact that accompanying symptoms of anorexia and vomiting are so characteristic of many other ills of infants.

The onset in infants is abrupt in contrast to the gradual onset in children and adults. To aid in the diagnosis of this young group the following symptoms are noted: fever, pulmonary signs and symptoms, anorexia, vomiting, restlessness, polyuria, weight loss and glycosuria. Additional important symptoms in the infant include recurrent enuresis and sticky diapers.

In older children the classical symptoms of polyuria, polyphagia, polydipsia, weight loss and glycosuria are usually present. Severe abdominal pain often accompanies approaching ketosis and simulates acute abdominal disease. The diffuse abdominal pain of diabetic acidosis recedes with the administration of insulin and parenteral fluids, while the pain of appendicitis or other abdominal disease persists. It is noted that the interval between onset of symptoms and diagnosis gradually increased in this study as the age of the patients increased. The average interval of three and six-tenths weeks in our study differs considerably from that of Danowski's series.[4] Danowski included 405 juvenile diabetics of all ages in whom the average interval between onset of the disease and diagnosis was eleven and seven-tenths weeks. This may be even more sharply contrasted to the longer interval of months or years before a diagnosis of diabetes mellitus is made in adults.[5]

The more common symptoms of polyuria, polydipsia, polyphagia and weight loss were less frequent in the twelve- to twenty-three-month age group. However, anorexia was more frequent in this youngest group. Discovery of diabetes was usually made by the routine diagnostic tests in an attempt to find a cause for the individual's loss of appetite, irritability, or during a severe infection.

Enuresis occurred in 6 per cent of our patients. We considered bed-wetting a symptom only in those infants and children who had been toilet-trained previously. This is in contrast to Danowski's series[4] in which this symptom was present in almost 40 per cent of 513 juvenile diabetics of all ages. In only two of our children was diabetes discovered on routine urinalysis. This low incidence is in notable contrast to the 32 per cent of patients discovered on routine urinalysis by other investigators.[4] The relation of hypoglycemia to the onset of diabetes mellitus in adults has been well reviewed and reported by Seltzer and associates.[6] However, this association has not been emphasized with regard to the juvenile diabetic. We observed two children whose onset of diabetes was diagnosed because of a hypoglycemic episode.

The incidence of acidosis in our series is considerably higher than the 10 to 18 per cent noted by others.[4,7] This can possibly be explained by the fact that many of the patients at the Children's Memorial Hospital are referred undiagnosed by other hospitals and nonstaff physicians. They enter because of severe vomiting, dehydration, or infection.

In forty patients (36 per cent) we found associated diseases. Thirty-three per cent of the entire group had an associated respiratory infection. Two patients had an exanthematous disease; one child had pyelitis, and another vaginitis. These figures are similar to those of other authors.[3,4]

It must be emphasized that diabetes mellitus in the twelve- to twenty-three-month age group may masquerade as pneumonia, gastritis with dehydration, and even meningitis. A routine urinalysis and blood sugar should be done whenever the possibility of diagnosing diabetes is entertained. While the onset of this disease is frequently associated with various infectious processes, they play no role in the etiology of our juvenile diabetics.

Situations of stress such as trauma, emotional problems, infections, obesity, and endocrine disturbances were not seen in our group. However, we are of the opinion that these aforementioned conditions do not precipitate diabetes mellitus, or necessarily precede it.

There is no definite relationship between the time of the year in relation to the onset of diabetes. Though most of our cases occurred in the fall and winter which is similar to some series,[4,8] it differs from others[9,10] in which a higher onset is noted in the spring and summer. Diabetes mellitus cannot be regarded as seasonal.

There was a family history of diabetes in 28 per cent of our patients. This figure is somewhat lower than that quoted by others.[3,4,11] The low incidence can be explained by the fact that our patients are quite young, and that there is a trend to increased familial incidence of diabetes as the juvenile patient grows older. This is attributable to the appearance of diabetes in distant relatives and siblings with the increasing age of the juvenile diabetic.

It is noteworthy that there was not a single case of a sibling with diabetes in this series.

Once the young diabetic is diagnosed and treated, he usually does well. The initial diabetic acidosis is treated with regular insulin and parenteral fluids. Antibiotics are prescribed for any co-existing infection. Oral feedings are gradually introduced, and when able the patient receives a specific weighed diet. At this time Lente insulin is usually administered.

The basic principle underlying the treatment of infants and children is thorough education of their parents. The parents should know the uses of the different insulins; diet prescriptions; how to perform urinalysis; how to recognize and prevent complications; and how to preserve the mental health of the child. Details of our routine management have been reported elsewhere.[12,13] The great majority of this age group of juvenile diabetics do exceptionally well, and are cooperative. They rarely require hospitalization for the usual childhood infections and contagious diseases. Activity is unrestricted, and they compete on an equal footing with their nondiabetic contemporaries.

SUMMARY

The records of 110 infants and children with onset of diabetes mellitus between one and five years of age were reviewed.

Sex distribution, average interval between onset of symptoms and diagnosis, presenting complaints, associated diseases, seasonal incidence, and family history were examined.

The diagnosis of diabetes mellitus in infants is rare. The diagnosis is seldom made because of the infrequency of the disease, its sudden onset, and because the accompanying symptoms of anorexia and vomiting are so characteristic of many other ills of infants. The onset in infants is abrupt in contrast to the gradual onset in children and adults. In older children the classical symptoms of polyuria, polyphagia, polydipsia, weight loss and glycosuria are usually present.

The basic principle underlying the home care and treatment of infants and children with diabetes mellitus is thorough education of their parents.

SUMMARIO IN INTERLINGUA

Diabete Mellite A Etates De Inter Un E Cinque Annos: Constatationes Al Tempore Del Declaration Del Morbo

Es presentate un revista del dossiers de 110 infantes e juveniles con diabete mellite a declaration a etates de inter un e cinque annos. Es discutite le distribution secundo le sexos, le intervallo medie inter le declaration del symptomas e le diagnose, le gravamines de presentation, le morbos associate, le incidentia saisonal, e le historias familial.

Le diagnose de diabete mellite in infantes es rar proque (1) le morbo mesme es rar, (2) su declaration es subite, e (3) le symptomas que accompania lo—i.e. anorexia e vomito—es characteristic de multe altere morbos de infantia. Le abruptitate del declaration del morbo in infantes contrasta con le declaration gradual in juveniles e adultos. In juveniles de etate plus avantiate, le symptomas classic de polyuria, polyphagia, polydipsia, perdita de peso, e glycosuria es usualmente presente.

Le base del custodia e del tractamento domiciliari de infantes e juveniles con diabete mellite es le education del parentes.

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Effect of Prolonged Administration of Glucagon in Guinea Pigs

Sydney S. Lazarus, M.D., M.Sc. (Med.), and Bruno W. Volk, M.D.,
with the technical assistance of
Patricia Lofaro, B.S., and Josephine McLeod, B.S., New York

The rat,[1-3] rabbit,[3] cat and dog[4] have been reported to be relatively insensitive to the hyperglycemic action of protracted glucagon administration. However, in one study large amounts of glucagon given to rats for a period of ten days have been shown to produce a diabetic effect.[5,6] There are only two reports which we know of concerning the effect of glucagon on pancreatic morphology. Kracht[7,8] noted involution of the pancreatic α cells and degranulation and hypertrophy of the β cells after prolonged administration of glucagon in the rabbit and rat. Salter and his colleagues[6] reported in two dogs, in whom daily administration of glucagon for one week produced transient hyperglycemia and mild glycosuria, either more intense granulation or degranulation and hydropic degeneration of the β cells as well as marked atrophy of the acinar cells. However, no α-cell changes were mentioned.

In a recent publication[9] we reported the effect of protracted administration of glucagon alone, or with subdiabetogenic doses of cortisone, on the blood sugar and pancreatic morphology of the rabbit. Glucagon was shown to cause a marked reduction in the number of identifiable α cells in the pancreas. In many instances partly degranulated or poorly stained α cells were observed as well as clusters of pyknotic nuclei with ill-defined cytoplasm which were thought to represent degranulated, somewhat involuted α cells. Marked hyperglycemia was maintained in the rabbit treated simultaneously with glucagon and cortisone, whereas glucagon alone produced only a mild transient hyperglycemia. In the hyperglycemic animals β-cell degranulation and glycogen infiltration of duct epithelium and of β cells were found. Furthermore, when diabetes was maintained over a long period, new β cell formation from ductular epithelium could be followed.

The present experiments are an extension of the previous work[9] and concern the possible effect of prolonged injections of glucagon on the pancreas and urinary sugar in the guinea pig.

Furthermore, the hormonally induced morphologic changes in the pancreas were compared with those produced by cobalt administration.

MATERIAL AND METHODS

Guinea pigs of either sex, weighing between 700 and 1,000 gm., were divided into three groups. Group A consisted of twelve animals, each of which received 0.33 mg. of glucagon* suspended in corn oil three times a day at 9:30 a.m., 4:30 p.m. and 12 midnight. Six of these animals expired. Of the others two were sacrificed at twenty-eight days, one at forty-two, one at ninety-five and two at 124 days. Group B consisted of six guinea pigs which received subcutaneously 30 mg./kg. of cobaltous chloride as 1 per cent solution for four consecutive days and were sacrificed on the morning of the following day. Group C consisted of twelve guinea pigs which served as untreated controls.

All animals were kept in metabolism cages and received Purina rabbit chow, as well as lettuce, carrots and tap water ad libitum. Daily twenty-four-hour urine specimens were collected for glucose determination.

The surviving guinea pigs were sacrificed by overdosage with sodium nembutal, and pancreatic tissue was fixed in Zenker formol solution. The blocks were embedded in paraffin and sections were stained by the following methods: iron hematoxylin, phosphotungstic acid hematoxylin, modified Masson trichrome,[10,11] a modification of the aldehyde fuchsin trichrome,[12] a modification of Gomori's chrome alum hematoxylin[11,13] and the Periodic Acid Schiff technic with a trichrome counterstain, controlled by diastase digestion.[14,15] The pancreata of the guinea pigs which died were not utilized for morphologic studies because of the rapid postmortem changes in the α cells.

From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, New York, and the Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

*Crystalline glucagon was supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, through the courtesy of Dr. W. R. Kirtley.

RESULTS

In the normal guinea pig the output of urinary reducing substances varied between 0 and 109 mg. in twenty-four hours. Cobalt-treated guinea pigs showed urinary glucose outputs similar to those of the normal. The guinea pigs treated with glucagon revealed a urinary glucose output varying from 1.5 mg. to as much as 2.6 gm. in twenty-four hours. However, a severe degree of glycosuria could not be maintained by glucagon administration.

Morphologically, the normal guinea pigs showed the usual cell types in the pancreas. The α cells were characterized by their perivascular location with deeply acidophilic and siderophilic rather coarse granules (figure 1). The large interlobular islets more frequently contained α cells than did the small islets. There were also present in the normal guinea pig islet some cells with faintly eosinophilic or unstained cytoplasm which were neither α, β or δ cells. Interacinar acidophilic and siderophilic cells, the so-called x-cells,[18,10,17] were readily identified.

The cobalt-treated animals showed, in varying degrees, typical lesions of the α cells (figure 2).[18,19] These consisted most characteristically of markedly enlarged vacuolated α cells which under low power gave the islets a cribriform appearance. These vacuolated α cells had intact nuclei which were frequently eccentric and showed strands of residual cytoplasm. Other α cells showed almost agranular cytoplasm or diminution in cytoplasmic granulation. Neither the β cells nor any other structures of the pancreas appeared to be altered.

The glucagon-treated guinea pigs sacrificed at twenty-eight days showed an apparent loss of α cells. On closer inspection of the islets it was seen that there were many cells with faintly acidophilic or siderophilic cytoplasm which were considered to be degranulated α cells. An occasional cell with faintly staining cytoplasm was also seen which could not be definitely identified as an α cell. The β cells were well granulated. At forty-two days there was also considerable loss of stainable α cells as well as, in this particular animal, an apparent increase in size and number of islets. The guinea pig treated for ninety-five days showed marked reduction in the cytoplasmic stainability of the α cells (figure 3). In these animals there was considerable islet hyperplasia and very moderate β-cell degranulation. The animals treated for 124 days had only infrequent, faintly staining α cells. In addition, there were frequently cells with foamy unstained cytoplasm which were thought to represent degranulated α cells (figure 4). The β cells were but moderately degranulated.

DISCUSSION

This study demonstrated that the prolonged administration of glucagon to guinea pigs causes a considerable diminution of pancreatic α-cell granulation which is most pronounced in the animals treated for the longest period. This lesion is somewhat similar to those previously observed in rabbits in which chronic glucagon administration also causes a decrease in identifiable α cells.[9] In contrast to the findings in the rabbit, however, no nuclear changes or cytoplasmic shrinkage were identified. These morphologic changes are, however, quite different from the punched-out destruction of α cells seen in the pancreas of guinea pigs treated with cobaltous chloride. The differences are assumed to be due to the fact that the latter is a noxious chemical agent which selectively destroys α cells, whereas glucagon is a physiologic substance which suppresses the secretory function of these cells. This suppression of secretory function is reflected in the reduction or total disappearance from these cells of cytoplasmic granulation. These findings strongly support the viewpoint that glucagon is a hormone produced by the α cells. They are analogous to those observed with other exogenously administered hormones which suppress endogenous secretion and cause ensuing atrophy of the glands of origin.

The prolonged administration of glucagon caused little or no alteration in β-cell granulation in the guinea pig. This is in accord with the very moderate and occasional glycosuria which appeared in these animals. The failure of these rather large dosages of glucagon alone to produce severe hyperglycemia is in keeping with results of other workers who have found that most animals are rather insensitive to its prolonged administration.[1-6] This finding, furthermore, does not support the idea expressed by some that excessive glucagon production may be a common cause of diabetes.[20]

SUMMARY

A study has been conducted on the effect of prolonged glucagon administration in guinea pigs, and the resultant pancreatic lesions were compared with those produced by cobaltous chloride. The administration of 0.33 mg. of crystalline glucagon suspended in corn oil three times daily for up to 124 days produced moderate transient glycosuria. Morphologic studies showed extensive degranulation of the α cells. The lesions, however, were quite different from the punched-out destruction of α cells seen in the animals treated with cobaltous chloride. The extensive degranulation observed in the α cells confirms previous observations in rabbits and sup-

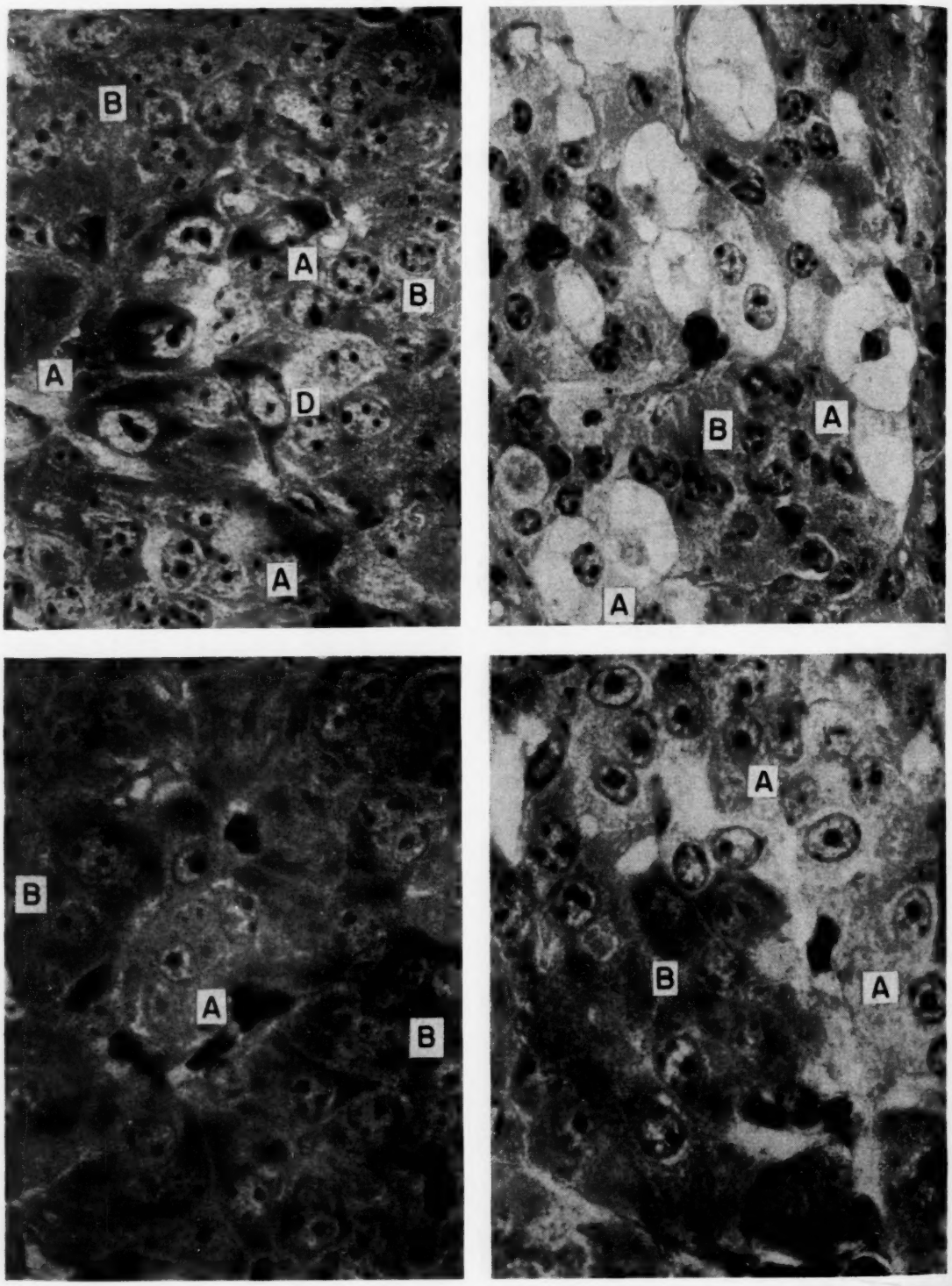
B
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FIG. 1. (Top left, page 296.) Islet from pancreas of untreated guinea pig showing groups of α cells (A) with dark granular cytoplasm, pale β cells (B) and a δ cell (D). Trichrome, ×1,280.

FIG. 2. (Top right, page 296.) Islet from pancreas of guinea pig treated with cobaltous chloride showing the typical vacuolization of α cells (A) and intact β cells (B). Aldehyde fuchsin, ×960.

FIG. 3. (Lower left, page 296.) Islet from pancreas of guinea pig treated with 0.33 mg. of glucagon in corn oil three times daily for ninety-five days. The α cells (A) show marked loss of cytoplasmic granulation. The β cells (B) are intact. Trichrome, ×1,280.

FIG. 4. (Lower right, page 296.) Islet from pancreas of guinea pig treated with glucagon for 124 days showing degranulation of α cells (A) and intact β cells (B). Aldehyde fuchsin, ×500.

ports the viewpoint that glucagon is a hormone produced by these cells.

SUMMARIO IN INTERLINGUA

Effecto Del Administration Prolongate De Glucagon In Porcos de India

Esseva effectuate un studio del effecto de prolongate administrationes de glucagon a porcos de India. In illo, le resultante lesiones pancreatic esseva comparate con le lesiones producite per chloruro cobaltose. Le administration de 0,33 mg de glucagon in forma crystallin e suspendite in oleo de mais, tres vices per die, durante periodos de usque a 124 dies, produceva moderate grados de glycosuria transiente. Studios morphologic revelava extense grados de disgranulation del cellulas alpha. Tamen, iste lesiones esseva multo differente ab le "punceate" destruction del cellulas alpha que esseva observate in le animales tractate con chloruro cobaltose. Le extense disgranulation observate in le cellulas alpha confirma previe observationes in conilios e supporta le idea que glucagon es un hormon producite per ille cellulas.

ACKNOWLEDGMENT

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Degenerative Lesions in Dogs with Experimental Diabetes

Henry T. Ricketts, M.D., C. E. Test, M.D., E. S. Petersen, M.D., H. Lints, M.D., Natalia Tupikova, and Paul E. Steiner, M.D., Chicago

The search for the cause of diabetic vascular disease in man is beset with two major difficulties. The first is that of separating the possible effects of constitution or heredity from those of diabetes itself. The question of genetic influences arises because of the occurrence of well-marked vascular lesions in some patients with very mild diabetes and their virtual absence in others with severe diabetes even of long duration, situations that would not be expected if the diabetic state alone were involved.

The second difficulty is that of determining conclusively whether good control of glycosuria prevents, and poor control favors, vascular complications in most if not all patients. The problem here is that it is almost impossible for any physician to know with certainty the habitual level of diabetic control that prevails in his patients for the ten to twenty years that are usually required for degenerative changes to appear.

It would seem that both obstacles might be overcome by the study of animals in which diabetes is induced experimentally, thus excluding genetic factors, and which can be observed daily under known conditions for long periods of time.

Several investigators have reported studies of the blood vessels in experimental diabetes, but in general, the number of animals has been small and results have been inconsistent.

Fisher[1] described severe calcific arteriosclerosis in a subtotally depancreatized dog between one and two years of age which was sacrificed after eight months of "intermittently" controlled diabetes. Dragstedt[2] reported arteriosclerosis in 1.2 per cent of normal dogs but in 15 per cent of dogs made diabetic by total pancreatectomy and maintained with insulin. The lesions were found within nine months of operation. They were observed much less frequently in partially depancreatized dogs. Chaikoff and Kaplan,[3] however, found no arteriosclerosis in two depancreatized dogs surviving for five years. Allen and Lisa[4] have reported necropsy findings in a Scotch terrier aged fourteen years with diabetes, induced by partial pancreatectomy, of twelve years' duration and treated with 30 to 40 U. of insulin daily. No disease of arteries, kidneys or retinae was discovered. Lukens and Dohan[5] reported glomerulosclerosis in a dog with largely uncontrolled, metapituitary diabetes of five years' duration. Conn[6] has observed similar changes in another such dog. In 88 per cent of subtotally depancreatized diabetic rats with uncontrolled glycosuria, Foglia et al.[7] found glomerular lesions which developed in two to twelve months and which resembled somewhat the diffuse form of human intercapillary glomerulosclerosis. Mann and co-workers[8] reported similar but apparently not identical glomerular lesions in untreated alloxan-diabetic rats, but no such lesions in alloxanized rats which did not become diabetic. Gerritzen and others,[9] on the other hand, found no vascular, including glomerular, lesions comparable to those of human diabetes in rats with alloxan diabetes of eleven months' duration, but the glomeruli of the diabetic rats did show marked swelling of some loops as compared with the glomeruli of alloxanized but nondiabetic control rats. Sudak et al.[10] observed sacculation of veins, and later arteries, in the cheek pouches of hamsters after ninety to 120 days of alloxan diabetes whereas normal animals, and four of ten alloxanized but nondiabetic animals studied at 365 days, had but few such changes.

A new approach to the problem of diabetic renal disease was suggested by the work of Rich, Berthrong and

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From the University of Chicago, Departments of Medicine and Pathology, Chicago, Illinois.

Bennett[11] who gave cortisone daily for two weeks to nondiabetic rabbits sensitized with horse serum and observed glomerular lesions "characterized by dilatations of capillary glomerular loops, focal necrosis of cells of loops, and the formation of large hyaline masses in the tufts. . . ." Others[12-14] have reported nodular glomerular lesions, some of them fatty, in nondiabetic rabbits treated with cortisone alone. Pursuing this lead, Becker,[15] working with Friedenwald, produced retinal microaneurysms and glomerular lesions "similar to those described by Kimmelstiel and Wilson" in alloxan-diabetic rabbits treated with ACTH.

Bloodworth and Hamwi[16] confirmed these findings with respect to the kidney in cortisone-treated rabbits, some of which were also alloxanized, but the lesions tended to disappear with time even while cortisone injections were continued.

The present study of long-term experimental canine diabetes is, so far as we can discover, the most extensive on record and the only one in which comparisons between the effects of good and poor control of glycosuria have been attempted.

MATERIALS AND METHODS

Beginning in 1947, approximately 120 young dogs, most of them beagles, were rendered diabetic with alloxan, subtotal pancreatectomy, anterior pituitary extract or a combination of these after preliminary biopsy of kidney and liver. Thirty-two, of which twenty were beagles, survived with active diabetes from 1 to 8.5 years. They were divided into two groups: In group I, glycosuria was as well controlled as possible and food and insulin were given twice daily; in group II, heavy glycosuria was encouraged and the animals were fed once daily and given insulin, when required to prevent undue weight loss, prior to each feeding. Each major group was divided into three subgroups which received measured, constant diets of high, medium and low fat content, respectively, with supplements of bone meal, cod-liver oil, brewer's yeast and dog meal or biscuits containing multivitamins. All dogs were housed in metabolism cages and weighed monthly. In well-controlled cases, urine collections were made twice daily and tested qualitatively for sugar, insulin doses being adjusted accordingly. In poorly controlled cases, aliquots of daily twenty-four-hour urine collections were pooled in consecutive three-day periods for each animal and similarly tested. Tests for proteinuria were made irregularly. In all cases, twenty-four-hour collections of urine were analyzed quantitatively for glucose[17] at least once weekly. Postabsorptive blood sugar levels[18] were determined every one to two weeks and postprandial levels irregularly in well-controlled cases; the interval for poorly controlled cases was two to three weeks. Serum concentrations of sodium, potassium, calcium, cholesterol and nonprotein nitrogen were determined from time to time by standard methods. Ultracentrifugal analyses for serum lipoproteins were carried out in 1953 through the kindness of Dr. Richard Jones. At least once a year blood pressure was measured by a direct method, six-lead electrocardiograms were taken and the eyes examined ophthalmoscopically with the help of an ophthalmologist. Cataracts were graded o to ++++ on the basis of extent and density.

In 1955 all remaining animals were killed with Nembutal intravenously, necropsy being performed immediately. In animals dying accidentally, post-mortem examination was begun within fourteen hours, usually sooner. Tissues examined both grossly and microscopically included eyes, pituitary body, brain, thyroid, lungs, heart, liver, spleen, pancreas, kidneys, gonads, thoracic and abdominal aorta and their large branches, and multiple sections of myocardium containing coronary arteries.

Flat preparations of the retinae were made and stained in each case according to the method of Friedenwald.[19] Other tissues were fixed in alcohol-formalin and in Bouin's solution, imbedded in paraffin and stained with hematoxylin and eosin. Additionally, sections of liver and kidney were stained with Mallory's azan, periodic acid followed by Schiff's reagent, Sudan III and Best's carmine reagents. Sections of arteries were also stained with Sudan III.

The microscopic slides were examined by two of us (H.T.R. and P.E.S.). Arteriosclerosis was graded o to ++++ on the bases of gross examination and the thickness and extent of intimal plaques, seen microscopically. Lesions of the renal glomeruli specifically searched for included thickening of Bowman's capsule; thickening of the intercapillary stroma; fibrosis, atrophy, hyalinization and lipidosis of the tuft; presence of nodules; and thickening and hyalinization of arteriolar walls. The prevalence of the lesions was estimated by determining the percentage of abnormal glomeruli in the PAS section in each case (the azan section had to be used in four cases), between 100 and 200 glomeruli being counted. In such counts, glomeruli within or adjacent to areas of obvious inflammatory changes or scarring were excluded.

Six normal beagles were kept as controls under the same housing and feeding (medium fat diet) conditions as the experimental animals. Seven "normal" old dogs, formerly pets of friends, and seven with spontaneous

diabetes* provided bases for comparison of certain pathologic aspects.

RESULTS

Only dogs which survived with diabetes for one year or more are reported. Owing to attrition, so few animals with long-standing diabetes were left in any of the dietary subgroups at the end of the observations that biochemical and pathologic differences, if any, between them cannot be evaluated. Experimental results, therefore, will be presented only as comparison between the two major groups with well controlled and poorly controlled diabetes, respectively.

Levels of diabetic control (table 2). Among thirteen dogs with well controlled diabetes the average urinary glucose excretion per twenty-four hours was 19 gm. and the average fasting blood sugar 148 mg. per 100 cc. Among nineteen dogs with diabetes under poor control, corresponding values were 71 gm. and 221 mg. per 100 cc. It is necessary to point out that efforts to reduce glycosuria to near zero in the well-controlled group resulted in excessive mortality from hypoglycemia, so that levels of urinary and blood glucose that were higher than ideal had to be accepted. The range of insulin dosage for well controlled dogs was 10 to 38 U. and for poorly controlled dogs, 0 to 40 U.

Serum lipid levels for all components examined were higher than normal in both diabetic groups and higher in poorly than in well controlled diabetes (table 3).

Miscellaneous chemical studies. No significant differences were found between normal and diabetic dogs, either well or poorly controlled, with respect to serum levels of sodium, potassium, chloride, calcium, phosphorus or nonprotein nitrogen. Proteinuria occurred consistently in two well controlled dogs, both alloxanized, and in six poorly controlled dogs of which four had been made diabetic with alloxan (including one with gross hematuria) and two by subtotal pancreatectomy and anterior pituitary extract.

Blood pressure was within normal limits in all animals and did not change appreciably in any one animal over the entire period of observation.

Electrocardiograms, while variable from time to time, showed no consistent change over the years.

Cataracts were present in 84 per cent of the thirty-two dogs. These were both nuclear and cortical, the latter frequently being radially arranged. Of the thirteen dogs with well-controlled diabetes, ten had opacities of 0 to ++ and three of +++ or ++++ severity. Of the nineteen with poorly controlled glycosuria, seven had opacities of 0 to ++ and twelve of +++ or ++++ severity. Cataracts appeared as early as six weeks after induction of diabetes in some cases. No dog of the fourteen in which diabetes was produced by alloxan escaped cataract, while seven of the eighteen in which diabetes was produced otherwise remained free. Alloxanized dogs which did not become diabetic did not show cataracts.

Retinal lesions were not found in any dog, either during life or in post-mortem preparations.

Arteriosclerosis. The only grossly visible lesions occurred in the thoracic and abdominal aorta. These were

TABLE 1

Types of dogs studied

Experimental diabetes for one year or more	
Alloxan	12
Subtotal pancreatectomy	10
Subtotal pancreatectomy + pituitary extract	8
Alloxan + subtotal pancreatectomy	1
Alloxan + pituitary extract	1
	32
Normal controls	6
Spontaneous diabetes	7
Old dogs	7

TABLE 2

Levels of diabetic control

	No. of dogs	Age at death (years)	Dur. diab. (years)	Urine glucose (gm./day)	Blood glucose (mg./100 cc.)
Good control	13	4.6 (2.0-8.5)	3.2 (1.0-8.0)	19 (11-33)	148 (98-171)
Poor control	19	4.6* (1.5-9.0)	3.8 (1.0-8.5)	71 (53-131)	221 (146-282)

*Excludes two dogs of unknown age.

TABLE 3

Serum lipid levels

	Max. cholest. since onset (mg./100 cc.)	Lipids October, 1953 (mg./100 cc.)			
		S_f 10-20	S_f 20-40	S_f 40-70	Total lipids
Good control six dogs	329 (279-386)	56 (26-95)	60 (5-97)	3 (0-5)	959 (848-1,043)
Poor control eight dogs	416 (325-614)	107 (39-257)	114 (60-194)	18 (3-73)	1,199 (915-1,490)
Normal six dogs	198 (134-289)	45 (6-130)	46 (17-82)	3 (2-7)	833 (485-1,535)

*Studies on these dogs have been reported previously.[20] Ages at death ranged from 5.5 to 15 yr., known duration of diabetes from one month to six years and maintenance insulin dosage, giving incomplete control of glycosuria, from 10 to 40 U. daily.

few in number in any animal and varied in severity from fine wrinkling of the intima to slightly raised, yellowish-white, longitudinal streaks. They were never as marked as those observed in long-standing human diabetes and senile individuals, and in most cases had to be searched for carefully. There were no distinct elevated plaques and no ulcers. Microscopically, the lesions of both the aorta and other vessels consisted of apparently fibrous thickening of the intima, often with rupture of the internal elastic lamella, and a few mononuclear leukocytes but with little or no lipid present in the fat stains. Thus, there was nothing resembling atherosclerosis. Calcification occurred very rarely.* Occasionally degenerative changes in the media, sometimes containing lipid, were seen. Except in the smaller renal arteries of one dog, there was no significant narrowing of the lumen. The few lesions of medium-size and small arteries involved mainly the carotid and thyroid arteries. Minimal coronary sclerosis was observed in three diabetic cases, two of them poorly controlled; in one alloxanized dog which did not develop diabetes; and in three of the normal group.

In general, the microscopic appearance of the plaques was the same in experimental, normal, ancient, and spontaneously diabetic dogs. Thirty-one per cent of dogs with well-controlled diabetes (average age 4.6 years), 63 per cent of those under poor control (average age 4.6 years) and 100 per cent of normal dogs (average age 7.4 years) showed some arteriosclerosis (table 4). Similarly but more intensively and extensively affected than either the experimental or normal group were 100 per cent of nondiabetic old dogs with an average age of 14.6 years and 86 per cent of spontaneously diabetic dogs, likewise old, with an average age of 11.2 years.

When the entire experimental group is divided into ages above and below 4.5 years, it is found that only 33 per cent of younger but 73 per cent of older dogs showed some arteriosclerosis (figure 1).

Among eighteen diabetic dogs less than 4.5 years of age (figure 2), only one of seven under good control, but five of eleven under poor control, had arterial lesions. There was no correlation with sex.

TABLE 4

Frequency of arteriosclerosis

	No. of dogs	Age at death (years)	Dur. diab. (years)	Per cent showing any sclerosis
Experimental				
good control	13	4.6 (2.0-8.5)	3.2 (1.0-8.0)	31
poor control	19	4.6* (1.5-9.0)	3.8 (1.0-8.5)	63
Normals	6	7.4 (7.0-8.5)	——	100
Old dogs	7	14.6 (11-18)	——	100
Spont. diab.	7	11.2 (5.5-15.0)	1.4 (0.1-6.0)	86

*Excludes two dogs of unknown age.

*Attention is called to a *normal* structure in the aortic ring below the attachment of the cusps which appears as a nodule staining deep purple with hematoxylin and eosin as does cartilage. This may be mistaken for pathologic calcification.

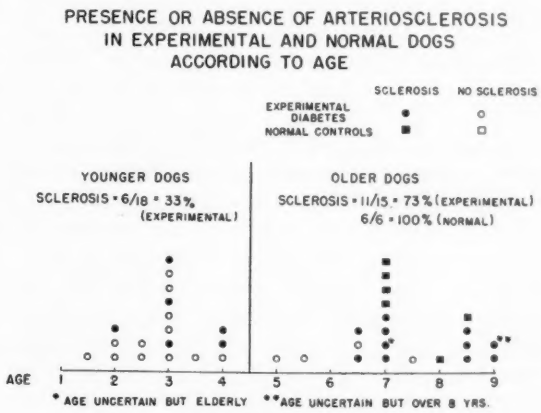
PRESENCE OR ABSENCE OF ARTERIOSCLEROSIS IN EXPERIMENTAL AND NORMAL DOGS ACCORDING TO AGE
SCLEROSIS
NO SCLEROSIS
EXPERIMENTAL DIABETES
NORMAL CONTROLS
YOUNGER DOGS
SCLEROSIS = 6/18 = 33% (EXPERIMENTAL)
OLDER DOGS
SCLEROSIS = 11/15 = 73% (EXPERIMENTAL)
6/6 = 100% (NORMAL)
AGE
1 2 3 4 5 6 7 8 9
* AGE UNCERTAIN BUT ELDERLY
** AGE UNCERTAIN BUT OVER 8 YRS.

FIGURE 1

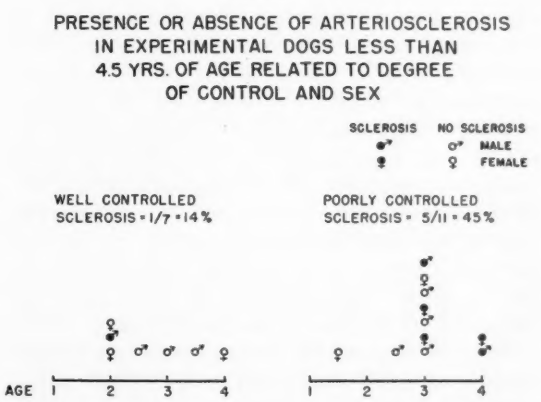
PRESENCE OR ABSENCE OF ARTERIOSCLEROSIS IN EXPERIMENTAL DOGS LESS THAN 4.5 YRS. OF AGE RELATED TO DEGREE OF CONTROL AND SEX
SCLEROSIS
NO SCLEROSIS
MALE
FEMALE
WELL CONTROLLED
SCLEROSIS = 1/7 = 14%
POORLY CONTROLLED
SCLEROSIS = 5/11 = 45%
AGE
1 2 3 4

FIGURE 2

Data for individual dogs with long-term experimental diabetes (five years or more) under good and poor control are given in table 5. All were 6.5 or more years of age. While the group with poor control seemed to have somewhat more arteriosclerosis than that with good control, especially in the aorta, the former, except for Dog 1, had no more than normal dogs of comparable age.

TABLE 5

Arteriosclerosis in long-term experimental diabetes and in normal controls

Dog No.	Sex	Age at death (years)	Dur. diab. (years)	Arteriosclerosis* Aorta	Coron.	Other
Good control						
101	m	8.5	8.0	0	0	+(3)
50	m	6.5	5.5	0	0	0
85	f	6.5	5.0	0	0	+(2)
Poor control						
1	m	9.0	8.5	++	+(1)	+(4)
161	m	8.5	7.5	+	0	+(2)
18	f	7.5	6.5	0	0	0
9	f	7.0	6.5	+	0	0
32	f	6.5	5.5	tr.	0	0
86	m	7.0	5.0	+	0	+(1)
Normals						
Six dogs aged 7-8.5 yr.:				+ 5/6†	0	+ 2/6†

*Degree of sclerosis graded 0 to ++++. Numerals after + signs indicate number of vessels showing sclerosis in microscopic sections.

†Indicates number of dogs with lesions out of total of six.

Renal lesions. Early in the course of the experiments it was found that ordinary doses of alloxan, especially when given repeatedly, result not only in tubular lesions, as is well known, but also in late glomerular damage.[21] All alloxanized dogs were therefore excluded in the studies of renal lesions here reported.

The typical nodular lesions of Kimmelstiel and Wilson were not seen in any case. However, thickening of the intercapillary stroma or of capillary walls themselves occurred in 13.3 per cent of the glomeruli of dogs with well controlled diabetes, in 11.2 per cent when diabetes was poorly controlled, and in 3.3 per cent among the normal dogs (table 6 and figure 3). Such thickening was usually focal and seldom involved a whole tuft. With respect to other glomerular abnormalities there were no significant differences in average frequency of occurrence between well and poorly controlled dogs or between either experimental group and normal dogs except for the presence of fat. Lipid deposits, usually at the glomerular root but frequently scattered throughout the tuft itself, were found in five of nine poorly controlled dogs (figure 4), an average of 21 per cent of the glomeruli being affected, but in only one dog each of the well controlled and normal groups. The fat droplets were small and often abundant but did not appear as nodules. In two dogs with excessive glycosuria of 5.0 and 5.5 years' duration, 78 and 98 per cent of the glomeruli, respectively, showed such lipidosis.

A few peculiar glomerular lesions (figure 5), first reported by us[20] in a dog with spontaneous diabetes, were found in an occasional animal of all groups, i.e.,

TABLE 6

Glomerular changes in nonalloxanized dogs

	No. of dogs	Per cent of glomeruli showing Thickened stroma or cap. wall	Focal hyaline deposits	Thickened arterioles	Misc. lesions*	Fat
Experimental good control	9	13.3 (1-25)	0.3 (0-2)	0.1 (0-1)	4.8 (0-13)	0.2 (0-1.5)
poor control	9	11.2 (1-43)	2.0 (0-16)	0.3 (0-2)	5.3 (0-13)	21 (0-98)
Normal	6	3.3 (1-4)	0.7 (0-2)	0	4.3 (0-15)	0.8 (0-5)

*Fibrosis or atrophy of tuft or thickened Bowman's capsule.

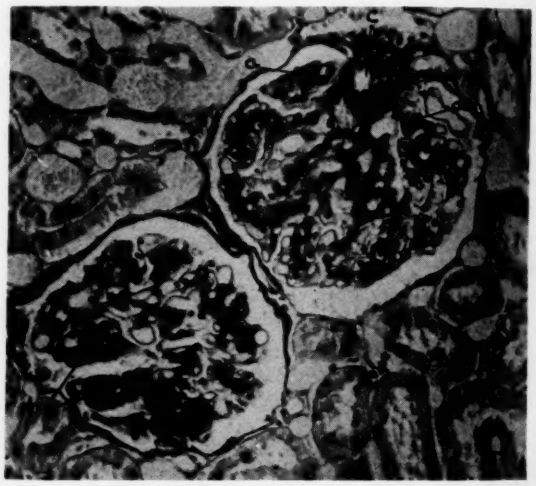

FIG. 3. Glomeruli of Dog 86 with uncontrolled diabetes of five years' duration. a. Thickening of stroma typical of that seen in 11.2 to 13.3 per cent of the glomeruli of diabetic dogs and in 3.3 per cent among normal dogs. b. Hyaline-like nodules; these were seen in only two dogs, both with uncontrolled diabetes. c. Thickened arteriole, rarely observed. PAS.

well and poorly controlled diabetics, normal dogs and nondiabetic old dogs. The involved glomeruli have been described as showing "one or more capillary loops forming lobular masses, sometimes involving the entire tuft, swollen by large pale cells, presumably endothelial cells. These are laden with lipid and apparently occlude the lumen of the capillary. Surrounding these cells or groups of cells are layers of connective tissue which is sometimes hyalinized. Parts, or entire tufts, are thus converted into fibrotic multiloculated spaces containing cells. These lesions vary in fibrous content from slight

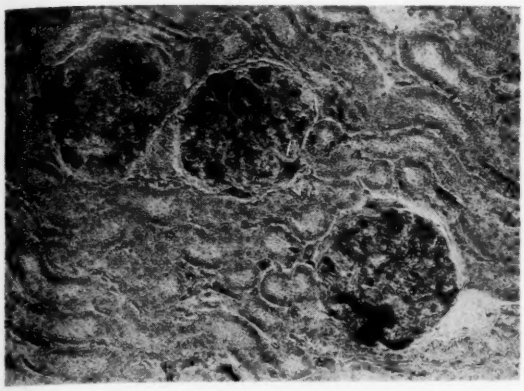

FIG. 4. Glomerular fat in Dog 32 with uncontrolled diabetes of 5.5 years' duration. Sudan III.

FIG. 5. Apparently nonspecific glomerular nodules seen occasionally in experimental and spontaneously diabetic dogs and old and middle-aged nondiabetic dogs. Mallory azan.

to very great. The latter type form fibrosed glomeruli which are similar to the usual fibrosed glomeruli. There is no change in blood vessels except that the glomerular lesions appear almost or completely avascular."[20] Similar lesions in a normal dog have been described by Fisher and Fisher.[22] Their significance is obscure.

Tubular abnormalities consisted of deposits of glycogen and excessive amounts of lipid in the great majority of dogs with poorly controlled diabetes. The tubules of normal animals and of those with well controlled diabetes showed but little fat and no glycogen.

COMMENT

The finding (table 4) of arteriosclerosis in 31 per cent of well controlled but 63 per cent of poorly controlled dogs would suggest that such lesions are favored by poor control were it not for the fact that 100 per cent of the normal dogs were similarly affected. Before concluding that the latter fact invalidates the former, however, it is necessary to consider the factor of age. Both of the experimental groups averaged 4.6 years at death and hence are comparable in this respect, whereas the normal controls averaged 7.4 years. Others[23,24] have reported that arteriosclerosis in the dog is more prevalent after the age of five years than before, and the data in figure 1 are confirmatory. Thus, it might be questioned whether a comparison of the experimental groups with a control group nearly three years older is fair. The question is given point by figure 2 which indicates that among diabetic dogs less than 4.5 years of age, despite the necessarily shorter duration of diabetes, arteriosclerosis occurred three times as frequently in those with excessive glycosuria as in those under good control. That the effect of aging on arteriosclerosis may mask the effect of diabetes is suggested by the fact that among older diabetic dogs, those under poor control, while perhaps having more vascular disease than those under good control, had little if any more than normal dogs of similar age (table 5).

Although differences in diabetic control, especially among younger animals, apparently were accompanied by differences in the frequency of the kind of arterial disease to which the dog is subject, it is clear that the lesions are qualitatively different from, and far less extensive and severe than, those observed in diabetic patients. This may be related to the fact that, while serum lipids were elevated in both well and poorly controlled dogs and more so in the latter, the greater proportion of the circulating lipids in the canine species, contrary to the human, is carried in the albumin-alpha globulin rather than in the beta globulin fraction of the lipoproteins.[25]

The development of cataract in 84 per cent of our diabetic dogs is in agreement with the experience of Chaikoff and Lachman[26] who reported this lesion in eight of ten depancreatized dogs surviving with diabetes for two years, but is at variance with the finding of Dohan and co-workers[27] that it occurred in only one of eight dogs with pituitary diabetes. We[20] have also found moderate to severe lenticular opacities in seven of eight dogs with spontaneous diabetes. An earlier impression[28] that cataract in dogs, as compared with other species, is uncommon when diabetes is produced by alloxan is

dispelled by its universal appearance in the alloxan-diabetic dogs of the present series.

The high incidence of cataract in this study is doubtless attributable to the fact that seven dogs marked for later good control were allowed to have considerable glycosuria for several weeks after induction of diabetes in order to insure the permanence of the diabetic state. In a number of these, the opacities tended to diminish after establishment of good control. Although the numbers of animals in the various dietary categories were too small to justify firm conclusions, there was a tendency for both the incidence and severity of cataract to be greater in dogs on a high carbohydrate than on a high fat diet. This conforms with the findings of Rodriguez and Krehl[29] in depancreatized and alloxanized diabetic rats.

The failure to find lesions in flat preparations of the retina of any dog leaves Becker,[15] working with rabbits, as the only investigator to have produced retinopathy in experimental diabetes. Retinal disease has been searched for but not found in dogs with spontaneous diabetes.[20]

Focal, occasionally generalized, thickening of the glomerular intercapillary stroma and of the capillary wall proper apparently characterizes long-term experimental diabetes in the dog, but good control of the degree here obtained does not prevent these lesions. They are also seen in many of the glomeruli of spontaneously diabetic dogs.[20] They resemble, in a milder form, the diffuse type of human intercapillary glomerulosclerosis,[30,31] but it is of course impossible to state that they are identical.

The most striking and numerous renal lesions were seen in Dog 86, aged seven years, with poorly controlled diabetes (subtotal pancreatectomy and anterior pituitary extract) of five years' duration (figure 3). On a high fat diet and with 4 U. of insulin daily, glycosuria averaged 88 gm. per day, blood sugar 234 mg./100 cc. and proteinuria one plus. The maximum serum cholesterol concentration was 450 mg./100 cc. Forty-three per cent of the glomeruli showed thickening of the stroma or capillary walls, sometimes diffuse, 16 per cent focal deposition of hyaline in the same areas or in the arterioles, 2 per cent thickening of arteriolar walls and 78 per cent lipidosis. Additionally, this was the only animal of the series with marked sclerosis of the smaller branches of the renal artery.

The presence of glomerular fat in the poorly controlled dogs constituted the greatest difference between the kidneys of this group and those of the well controlled and normal groups. There was no correlation with serum lipid concentrations. Among eight spontaneously diabetic dogs, for the most part poorly controlled, from zero to 68 per cent of the glomeruli contained fat.[20] It is of interest that Wilens[32] and co-workers have reported a considerably higher incidence of glomerular lipidosis among patients with intercapillary glomerulosclerosis than among those with other forms of renal disease. Likewise, Hartroft[33] has described glomerular "fat emboli" in 75 per cent of diabetic patients with Kimmelstiel-Wilson lesions but in only 15 per cent of diabetic patients without such lesions. It is uncertain whether fat deposition precedes or follows other manifestations of glomerular damage.

It is evident that for the most part the sequelae of long-term experimental diabetes in the dog do not bear a close resemblance, either qualitatively or quantitatively, to those seen in patients. The reason for this can only be conjectural at present: (1) Perhaps it is a matter of species difference. (2) Perhaps genetic factors, doubtless absent in the experimental diabetes of animals, determine the development of degenerative disease in man just as they largely determine the presence of diabetes. There is evidence, however, that in what might be termed human experimental diabetes, surgical removal of the pancreas or its destruction by disease in patients with no known diabetic ancestry may result in premature arteriosclerosis[34] and the nearly specific entity of retinopathy with microaneurysms.[35–37] It is a reasonable assumption that in such cases the diabetic state itself, not heredity, is the moving force. If so, one can only conclude that the dog responds to that state differently, less intensely or after a longer duration of diabetes than was obtained in the present observations. (3) It is possible that degenerative disease is a function of the absolute number of years over which the tissues are exposed to the metabolic defect rather than the years of exposure in relation to life span. Thus, it might take ten to twenty years of diabetes in the dog, as it does in man, rather than one third of the normal life expectancy, to evoke a comparable degree of vascular disease. However, the finding by others[1,2,5–8] of lesions in diabetic animals, especially in the kidneys, after relatively short periods of time renders this possibility unlikely.

Finally, the fact that such vascular lesions observed in experimental diabetes have not been proved histologically identical with those of human diabetes should not trouble us too much. It would be unreasonable to expect the tissues of different species to respond to an abnormal situation in precisely the same way. The important thing is that vascular damage of some sort has been shown to occur in the artificially induced diabetes of several species, including man. This is enough to show that hereditary predisposition is not essential.

SUMMARY

Thirteen dogs with well controlled and nineteen with poorly controlled experimental diabetes were observed for 1 to 8.5 years. In the well controlled group the blood sugar averaged 148 mg./100 ml. and the daily urinary glucose was 19 gm., while in the poorly controlled group these values were 221 mg./100 ml. and 71 gm. Serum lipoprotein and cholesterol levels were higher than normal in both diabetic groups and higher in poorly than in well controlled diabetes.

Mild arteriosclerosis, of the fibrous rather than the atheromatous type, was present in 31 per cent of dogs with well controlled diabetes, in 63 per cent of those under poor control and in 100 per cent of normal dogs, the latter, however, averaging three years older than the experimental groups. The effect of age on arteriosclerosis seemed evident in all groups and might have masked the effect of diabetes. This possibility is supported by the fact that of eighteen diabetic dogs less than 4.5 years old at death, only one of seven with well controlled diabetes, but five of eleven with poorly controlled diabetes, showed arteriosclerosis.

Thickening of the intercapillary stroma and of the capillary walls of the renal glomeruli was more frequent in both diabetic groups than in normal dogs, but there was no relationship with the degree of diabetic control. Poorly controlled dogs, however, showed a far higher incidence of glomerular fat deposits. No typical Kimmelstiel-Wilson nodules were found in any animal. Flat preparations of the retina revealed no lesions. Cataracts occurred in 84 per cent of all dogs, being more common and severe in those under poor control.

It is evident that the sequelae of long-term experimental diabetes in the dog do not closely resemble, either qualitatively or quantitatively, those seen in man. Nevertheless, the data indicate that in younger animals arteriosclerosis is more frequent in poorly controlled diabetes and that in dogs of all ages long continued, excessive glycosuria leads to glomerular lipidosis.

SUMMARIO IN INTERLINGUA

Lesiones Degenerative In Canes Con Diabete Experimental

Dece-tres canes con ben stabilisate e dece-nove con mal stabilisate diabete experimental esseva observate durante inter 1 e 8,5 annos. In le ben-stabilisate gruppo le sucro del sanguine habeva un valor medie de 148 mg per 100 ml, e le glucosa diurne del urina esseva 19 g, durante que le valores correspondente pro le mal-stabilisate gruppo esseva 221 mg per 100 ml e 71 g. Le nivellos de lipoproteina e de cholesterol in le sero esseva plus que normal in ambe le gruppos diabetic e plus alte in mal- que in ben-stabilisate diabete.

Leve grados de arteriosclerosis—del typo fibrose plus tosto que atheromatose—esseva presente in 31 pro cento del canes con ben-stabilisate diabete, in 63 pro cento del canes con mal-stabilisate diabete, e in 100 pro cento de un gruppo de controlo de canes normal, sed le etate medie in iste ultime gruppo esseva tres annos plus que in le gruppos experimental. Le effecto del etate super le arteriosclerosis esseva evidente in omne le gruppos e pote ben haber mascate le effecto del diabete. Iste possibilitate es rendite plus probabile per le facto que inter le 18 canes que moriva a etates de minus que 4,5 annos, arteriosclerosis esseva incontrate in solmente un del septe con ben-stabilisate diabete sed in cinque del dece-un con mal-stabilisate diabete.

Spissification del stroma intercapillar e del pariete capillar del glomerulos renal esseva plus frequente in ambe le gruppos diabetic que in canes normal, sed nulle correlation esseva constatate con le grado del stabilisation de diabete. Le gruppo mal-stabilisate, tamen, monstrava un multo plus alte incidentia de depositos de grassia glomerular. Nulle typic nodulos de Kimmelstiel-Wilson esseva trovate in ulle del animales. Preparatos plan del retina revelava nulle lesiones. Cataractas occurreva in 84 pro cento de omne le canes e esseva plus commun e plus sever in le mal-stabilisate gruppo.

Il es evidente que le sequellas de chronic diabete experimental in canes non resimila intimemente—in quantitate o in qualitate—le sequellas vidite in diabeticos human. Nonobstante, le datos indica que in plus juvene animales arteriosclerosis es plus frequente quando le diabete es mal stabilisate e que in canes de omne gruppos de etate, alte grados de longemente continuate glycosuria resulta in lipidosis glomerular.

ACKNOWLEDGMENT

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There are innumerable potential interactions between hereditary and environmental influences. It should be kept in mind that not all inhibitors are under genetic control. In addition it should be recalled that many enzymes have a coenzyme, or prosthetic group, as a necessary adjunct to the protein core. Although the protein core is determined by a gene, the coenzyme is often of vitamin origin, and is thus, in man, at least, environmentally conditioned. It is conceivable, therefore, that an enzyme which in one individual is inhibited by a genetically produced substance may in another individual be inhibited by an environmentally provided agent. Similarly, an enzyme which in one person is rendered dysfunctional by a genetic defect of the protein core, or apoenzyme, may in another person be inactivated by an environmental lack of the coenzyme. As a result of these and other exogenous effects, "phenocopies" may be produced, mimicking the genetic conditions. For example, nutritional siderosis appears to be a phenocopy of genetic hemochromatosis.

Laurence H. Snyder, in "Fifty Years of Medical Genetics," from *Science*, Jan. 2, 1959.

Metabolism of Insulin-I [131]

Studies in Isolated, Perfused Rat Liver and Hind-limb Preparations

Glenn E. Mortimore, M.D., Frank Tietze, Ph.D., and DeWitt Stetten, Jr., M.D., Ph.D.
Bethesda

The incubation of insulin with slices, homogenates or extracts of mammalian tissues has been shown to result in its biological inactivation and proteolytic degradation.[1-5] Inasmuch as crude extracts of liver have shown some specificity for insulin,[4,6] the view that a specific insulin degrading system, termed by Mirsky "insulinase," may exist has been proposed.[4] Although detectable levels of insulin protease are widely distributed, the greatest concentrations are found in homogenates of liver and to a somewhat lesser extent in kidney.[1] Muscle, a prime target of insulin, appears to contain very little of such activity in its extracts.[2] The rapid destruction of insulin in the intact animal could be attributed to hepatic and perhaps renal activity. However, such an assumption presupposes that circulating insulin has access to intracellular proteases of liver and kidney, and that "insulinase" activity is not an artifact of sliced and homogenized preparations. Since all endogenously produced insulin must pass through liver prior to reaching skeletal muscle and other target organs, the consequences of this passage are of considerable interest.

The purpose of this communication is to report the results of experiments designed to assess the relative magnitude of iodoinsulin degradation by perfused isolated, intact rat liver and hind-limb. Since at least the appearance of cellular integrity was well sustained, it is hoped that the present results more closely approximate reactions in normal tissues than do those obtained from slice and homogenate experiments.

EXPERIMENTAL

Perfusion technic. Fed, male Sprague-Dawley rats, weighing between 170 and 190 gm., were used as liver and hind-limb donors in the perfusion experiments to be described. The livers, which averaged 7.9 gm. in weight, were prepared for perfusion as follows: After etherization the rat's abdomen was widely opened and a small cannula, carrying oxygenated blood, was inserted and tied into the portal vein, care being taken not to disturb the bile duct. The chest was then opened and a cannula of larger diameter was placed in the inferior vena cava through an incision in the right atrium. This served to carry the effluent "venous" blood back into the oxygenating flask. To prevent loss of perfused blood by retrograde leakage, the inferior vena cava was ligated between the liver and right kidney. After an interruption of hepatic blood flow for a matter of one or two minutes, it was thus possible to establish a closed, isolated circulation.

Hind-limb preparations were perfused through a cannula tied into the abdominal aorta slightly above its bifurcation. Since it was not possible to collect the perfusate quantitatively from the inferior vena cava because of anastomotic connections with the vertebral veins, the operated animal was cleanly bisected just above the aortic cannulation, the hind-limb quarters were inverted, and the effluent blood was allowed to drip into a small funnel and thence to the oxygenator. All viscera except the urinary bladder, testes, prostate and seminal vesicles were removed. The hind-limbs ranged between 60.5 and 62.8 gm. in weight.

The perfusing blood volume of 50 ml. was collected immediately before the above procedure by exsanguinating five or six mature Sprague-Dawley male rats from the abdominal aorta, drawing the blood into heparinized syringes.

The effluent or "venous" blood entered the upper end of the perfusion oxygenator, a sloping glass cylinder 35 x 9 cm., which was rocked about its long axis through a 90° arc at eighty cycles per minute. The blood which collected at the lower end of the cylinder was filtered through nylon gauze and peristaltically pumped through Tygon tubing to the "arterial" cannula. The gas mixture, 95 per cent O_2—5 per cent CO_2, saturated with H_2O, flowed rapidly through the cylinder. In most experiments the blood flow was 7.0 ml./min.,

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creating a pressure gradient of 17—32 mm. Hg across the liver and 63—75 mm. across the hind-limbs. The entire perfusion procedure was conducted in a constant-temperature room at 37° C.

Treatment of blood samples. Individual 6 ml. samples were withdrawn from the perfusion vessel at 7.5, 15, 30 and 60 minutes after iodoinsulin was added. These samples, together with a zero-time control, were treated as follows: Aliquots of whole blood and plasma were directly assayed for radioactivity. One milliliter of plasma was diluted with 3.0 ml. of water and then 4.0 ml. of 10 per cent trichloroacetic acid (TCA) were added to precipitate the proteins. Five milliliters of the resultant clear supernatant fluid were removed for counting. All counting was performed in a well-type scintillation counter. From these data and the packed-cell volume it was possible to compute, for each milliliter of blood, the radioactivity contained in whole blood, in the cell fraction and in the TCA-soluble and precipitable fractions of plasma. For comparison all values were normalized, setting the whole blood radioactivity at zero time equal to 100 c.p.m./ml. blood.

Iodoinsulin was obtained from the Abbott Laboratories, Oak Ridge, and after dialysis contained not less than 97 per cent trichloroacetic acid (TCA)-precipitable radioactivity. The estimated degree of iodination was 1.0 atom of iodine per molecule of insulin (6,000 m.w.). Iodoinsulin preparations similar to those used in this study have been reported to retain full hypoglycemic activity.[7] The iodoinsulin was diluted with a solution containing 0.9 per cent saline and 0.5 per cent bovine serum albumin, and was then dialyzed at 4° C. against 0.9 per cent saline for twelve hours. Further dilution with the saline-bovine albumin solution was made just before use to achieve a final iodoinsulin concentration of 1 μg. per ml.

Paper chromatography. Unidimensional descending chromatography was carried out on 8 x 22½-inch strips of Munktell No. 20/150 filter paper[8] with the organic phase of a 3:1:4 mixture of n-butanol:acetic acid:water.[9] This system was chosen on the basis of its ability to resolve the components of a mixture consisting of insulin, 3-iodotyrosine, 3,5-diiodotyrosine, and inorganic iodide. No evidence has been found for the lability of organically bound iodine in this system. In more recent unpublished experiments we have obtained superior resolution of the above mixture with the butanol-ethanol-ammonia system of Tong and Chaikoff.[10]

After thorough air drying of the chromatogram, appropriate strips 1⅜ inches in width were cut lengthwise and scanned for radioactivity in a recording gas-flow paper strip counter.[11]

RESULTS

In all the experiments reported the perfused livers retained their normal moist, red-brown appearance and there was no evidence of swelling. Histologic examination* of a perfused liver following a typical experiment revealed generally normal-appearing cells except for occasional small, well-demarcated areas suggestive of localized ischemia. Oxygen extraction, denoted by marked darkening of the effluent blood, continued throughout the sixty-minute perfusion period and in one experiment, where the duodenum was cannulated, the continuous secretion of bile was observed.

In five experiments 1 μg. of iodoinsulin was added to the perfusion reservoir, and the rate of degradation followed by the changing distribution of radioactivity between the plasma TCA-soluble and precipitable fractions (figure 1). The initial concentration of iodoinsulin was 0.02 μg./ml. of blood, an amount which, it was hoped, might roughly approximate physiological levels in rat portal vein blood. There was a rapid decline in TCA-precipitable radioactivity, reaching a mean level of 29.4 per cent by sixty minutes (table 1). By contrast, the rate of appearance of plasma TCA-soluble activity was initially very slow and by 7.5 minutes there was only a negligible rise. The initial rapid capture of plasma TCA-precipitable activity by the liver with the delayed release of plasma TCA-soluble radioactivity was

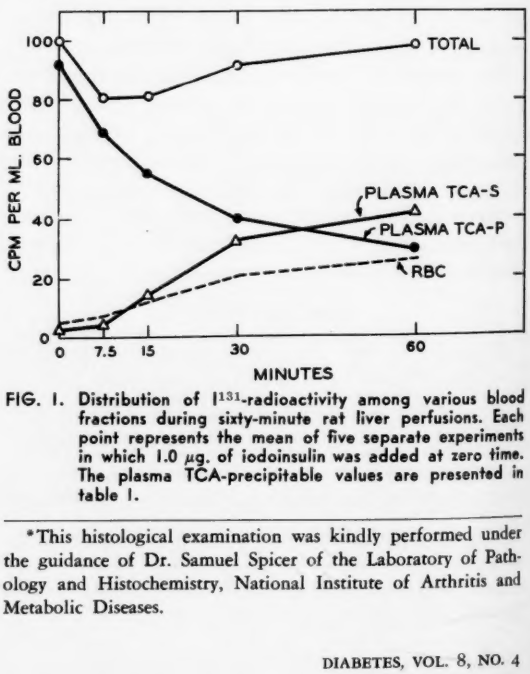

FIG. 1. Distribution of I^{131}-radioactivity among various blood fractions during sixty-minute rat liver perfusions. Each point represents the mean of five separate experiments in which 1.0 μg. of iodoinsulin was added at zero time. The plasma TCA-precipitable values are presented in table 1.

*This histological examination was kindly performed under the guidance of Dr. Samuel Spicer of the Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases.

TABLE 1

Decrease of plasma TCA-precipitable radioactivity in c.p.m./ml. blood with time in five liver perfusions. One microgram of iodoinsulin was added at zero time

Experiment	16	17	19	20	77	Mean
Minutes						
0	89.0	93.8	89.0	93.8	94.4	92.2
7.5	69.3	76.9	65.9	70.0	61.6	68.7
15	58.8	56.3	53.4	53.5	50.7	54.5
30	39.6	38.4		41.6	38.0	39.4
60	26.9	27.2		32.5	30.8	29.4

undoubtedly responsible for the dip in the whole blood radioactivity concentration shown in figure 1. Later, as the rate of appearance of TCA-soluble material increased, total blood radioactivity approached its initial value. Although a possibility exists that this dip was related in part to a dilution by the fluid volume of the liver, we feel it is unlikely in view of our failure to detect a significant fall in total radioactivity in later experiments employing higher concentrations of insulin. Radioactivity associated with the cellular fraction of blood (labeled RBC in figure 1) in general followed the plasma TCA-soluble curve and in the latter half of the perfusion amounted to about 60 per cent of the plasma TCA-soluble activity, a distribution in substantial agreement with that of Berson.[12] It would appear that the RBC radioactivity is derived from the TCA-soluble material of plasma. With one exception all of these experiments were conducted with a uniform rate of blood flow of 7.0 ml./min., a rate which approximates reported estimates of splanchnic[13] and hepatic blood flow[14] in rats. When the flow rate was decreased to 3.8 ml./min. in one experiment no change occurred in rate of distribution of radioactivity among the various fractions and it was concluded the blood flow generally employed was great enough to provide adequate hepatic flow and prompt mixing of blood among the various components of the perfusion system.

The possibility that insulin degradation might occur in the plasma per se by virtue of an elution of intracellular proteolytic enzymes from the liver was dispelled by the following experiment. Plasma from a control sixty-minute rat liver perfusion was incubated with a trace amount of iodoinsulin for 1 hour at 37° and the degree of degradation compared with a similar incubation employing fresh rat plasma. No significant degradation occurred in either sample. Although the livers were isolated only in a "vascular" sense there was no extrahepatic loss of radioactivity via bile secretion, lymph drainage or diffusion since the radioactivity added could be quantitatively recovered at the end of the perfusion (table 2).

TABLE 2

Recovery of radioactivity in c.p.m. (not normalized) in four rat liver perfusions where the liver was assayed for radioactivity

Experiment	16	17	20	77
Remaining in blood	297,920	190,400	196,512	734,080
Removed by sampling	142,620	95,400	99,018	339,480
Liver	24,240	14,580	17,430	65,400
Total recovered	464,780	300,380	312,960	1,138,960
Total added	465,500	295,000	323,050	1,169,500
Per cent recovered	100	102	97	97.5

If it is assumed that: (a) Iodoinsulin is destroyed by the liver at a rate proportional to its instantaneous concentration in the blood presented to the liver, and (b) the recycling rate is sufficient to bring about essentially "instantaneous" mixing between the reservoir blood and the blood in the liver, the rate of decline in concentration of iodoinsulin in blood, $-dC/dt$, will be inversely related to the volume, V, of the cyclically perfusing blood.

$$-V\frac{dC}{dt} = k(C - C_\infty) \qquad (1)$$

Where V = total blood volume, in milliliters, C = concentration of iodoinsulin-I^{131} in c.p.m. per ml. of blood, C_∞ = asymptote approached by C after prolonged perfusion and k = reaction velocity constant, differing from the traditional first order constant in having dimensions of milliliters per minute. This constant k can be shown* to equal the product of the traditional first order constant and the volume of blood contained in the liver. Upon integration, equation (1) becomes:

$$k = \frac{2.3V}{\Delta t}\log\frac{C_a - C_\infty}{C_f - C_\infty} \qquad (2)$$

where C_a and C_f are initial and final concentrations over a time interval Δt.

An extrapolation from data in table 1 gives a mean value of 29.2 c.p.m. per ml. for C_∞. The physical meaning of this quantity is not entirely clear. It might signify that of the "iodoinsulin" initially added, some 29 per cent had been altered in preparation so as to be no longer susceptible of attack by liver. Alternatively, it might represent a stable fragment derived from iodoinsulin by hepatic proteolysis but of sufficient molecular

*The authors are indebted to Dr. J. Z. Hearon of the National Institute of Arthritis and Metabolic Diseases for his valuable assistance in the present mathematical treatment. A rigorous development of this and related kinetic problems will shortly be published by Dr. Hearon.

size to retain the property of TCA-precipitability.

The form of equation (2) permits adjustment to be made for the stepwise decrease in V which resulted from the sampling procedure. For each successive time interval the value for k has been computed (table 3) and a mean value of 3 ml./min. has been obtained. This quantity has a superficial similarity to "clearance" of the renal physiologists and indicates that of the 7 ml. of blood pumped through the liver each minute, iodoinsulin was extracted from 3 ml., or approximately 40 per cent.

The mean distribution of radioactivity in blood fractions from five rat hind-limb perfusions is shown in figure 2. The same experimental design used in the liver perfusions was employed in each instance. When compared with the liver perfusions (figure 1) one important difference was recognized, namely, the much slower rate of appearance of the TCA-soluble product in the hind-limb perfusate. This difference is magnified several-fold when the same comparison is made by weight of tissue. Although the rate of fall of TCA-insoluble activity appears to be comparable to that observed in the liver experiments, it is suspected that much of this decrease is related to dilution of the substrate by the large mass of perfused tissue. In a separate determination of the *inulin* space of a 60-gm. hind-limb preparation a 30 per cent reduction in the circulating concentration of *inulin* by dilution with tissue fluids was observed. Because neither the rate nor volume of dilution was measured directly in the above hind-limb experiments, it was not possible to compute the rate of disappearance of TCA-precipitable radioactivity as was done for the liver perfusions. A comparison of the time-concentration curves of the two groups of perfusions by inspection, however, leaves little doubt that the rate of uptake by hind-limb tissue is significantly smaller than by liver.

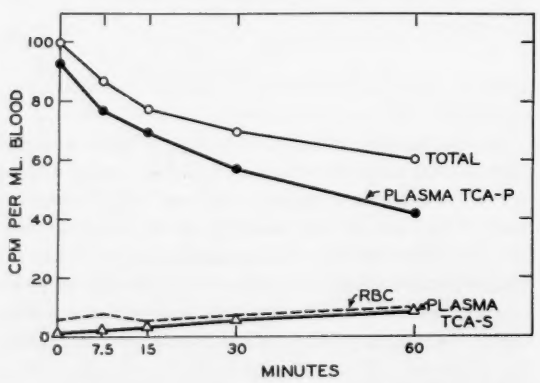

FIG. 2. Distribution of I^{131}-radioactivity among various blood fractions during sixty-minute rat hind-limb perfusions. Each point represents the mean of five separate experiments in which 1.0 μg. of iodoinsulin was added at zero time.

TABLE 3

Determination of k (ml./min.) for the hepatic removal of plasma TCA-precipitable radioactivity in five liver perfusions. Equation (2) in the text was applied to each time interval between samples. C∞ is the extrapolated asymptotic concentration of 29.2 c.p.m. per ml. blood

Time of sampling (min.)	C (mean plasma TCA-precipitable radioactivity in c.p.m./ml. blood. From table 1)	C — C∞ (c.p.m./ml. blood)	Δ t (min.)	V (ml.)	k (ml./min.)
0	92.2	63			
7.5	68.7	39.5	7.5	50	3.11
			7.5	44	2.63
15	54.5	25.3			
			15	38	2.30
30	39.4	10.2			
			30	32	4.16
60	29.4	0.2		mean	3.05

In view of the uncertainty regarding the mechanism of action of the sulfonylurea hypoglycemic agents and the claim by some that they will inhibit the degradation of insulin,[15] we thought it of interest to explore such an effect in isolated liver preparations. The sodium salt of tolbutamide,* dissolved in distilled water, was given intraperitoneally at a level of 225 mg. per kg. body weight both to the blood-donor and "liver"-donor rats three hours prior to the experiment. This amount of the drug was sufficient to cause a 40 per cent lowering of blood glucose in control animals. In figure 3 are shown the results of perfusing 1 μg. of iodoinsulin in a manner identical to the control liver perfusions. Since the time-concentration curves for the various fractions in figure 3 are essentially superimposable on those in figure 1, it may be concluded that tolbutamide did not (directly or indirectly) alter the fate of iodoinsulin.

Evidence that the degradation of iodoinsulin and native insulin may share a common reactive step is presented in figure 4. It may be seen that the perfusion of mixtures of 10, 100 and 1,000 μg. of crystalline insulin separately with 1 μg. of iodoinsulin was associated with decreasing rates in the cumulative hepatic removal of plasma TCA-precipitable radioactivity. This falling rate of removal of radioactivity in the presence of increasing amounts of

*A generous amount of sodium tolbutamide (Orinase) was kindly supplied by Dr. C. J. O'Donovan of The Upjohn Company, Kalamazoo, Michigan.

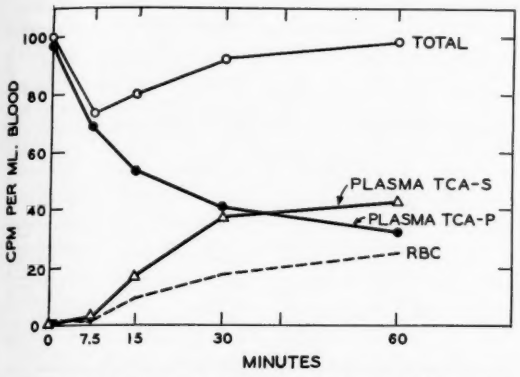

FIG. 3. Lack of effect of tolbutamide (Orinase) on the capture and degradation of iodoinsulin by perfused rat liver. Experimental conditions were otherwise identical to those whose results are depicted in figure 1. Each point represents three separate experiments.

native insulin is indicative of substrate competition for a rate-limiting step or steps. Such evidence, although inconclusive, is compatible with the thesis that the pathways of iodoinsulin and native insulin degradation are identical. Similar effects with intact mice,[20] eviscerated nephrectomized rabbits[71] and liver extracts,[4,18] have been previously reported.

An attempt was made to follow with paper chromatography the degradation of iodoinsulin during liver perfusion. Samples of plasma removed during the course of a perfusion in which 10 μg. of iodoinsulin had been introduced, were spotted on paper and chromatographed

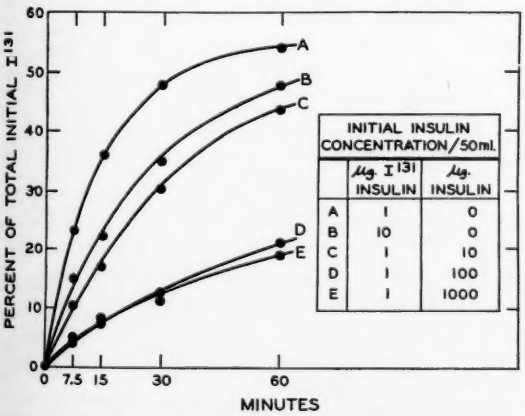

FIG. 4. Cumulative net removal of plasma TCA-precipitable I^{131} by perfused rat liver at various insulin concentrations. In calculating the values for each point a correction was made for the prior removal of radioactivity by sampling. The points of curves C, D and E represent means of two experiments each; B represents one experiment; A represents the mean of the five experiments shown in figure 1.

as described previously. A reciprocal change with time in the two major peaks on the radio chromatogram, bearing mobilities identical to insulin-I^{131} and iodide-I^{131} may be noted (figure 5). It will be seen in this figure that a relatively constant fraction of radioactivity of unknown nature remained fixed at the origin. This phenomenon, encountered in all similar chromatograms, is undoubtedly associated to some extent with the presence of a plasma protein of low mobility in this system. Since liver is known to contain a specific deiodinase for mono- and diiodotyrosine,[19] it seemed reasonable to assume that a substantial portion of this iodide-I^{131} was derived from iodotyrosine-I^{131} subsequent to the hydrolysis of iodoinsulin. Despite its unlikelihood, the possibility of deiodination occurring before the liberation of iodotyrosine—from peptides or iodoinsulin, itself—was investigated.

Ten micrograms of iodoinsulin were perfused in the presence of unlabeled mono- (MIT) and diiodotyrosine (DIT). Ten milligrams each of MIT and DIT were added just before the iodoinsulin, and the same amount adminis-

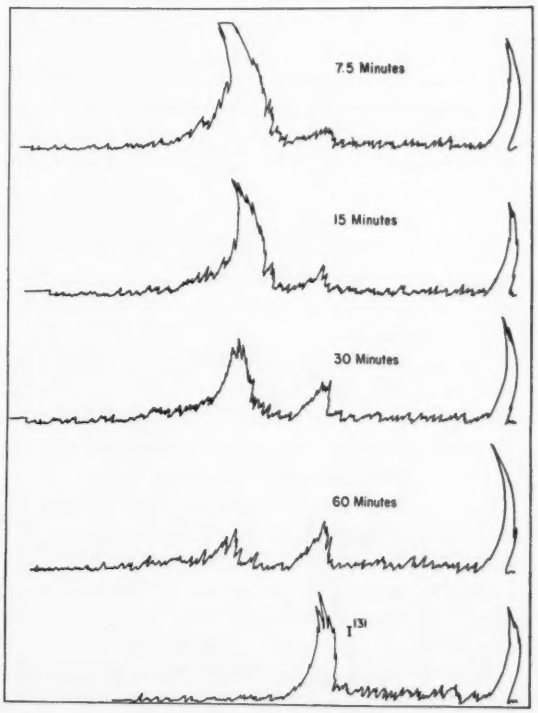

FIG. 5. Radiochromatograms of plasma sampled at the various times indicated during a rat liver perfusion in which 10 μg. of iodoinsulin was administered at zero time. The point of origin for each strip is the peak on the right. The peak which decreases with time on the left has the mobility of iodoinsulin.

tered by a motor-driven syringe continuously during the sixty-minute perfusion period. Plasma samples were then chromatographed in the same system previously described. As shown in figure 6 the iodoinsulin peak diminished at virtually the same rate as in figure 5, but the appearance of iodide-I^{131} was abolished. A small peak having the mobility of MIT was observed and is especially prominent in the thirty-minute sample (figure 7). These findings are compatible with the assumption that mono- and/or diiodotyrosine-I^{131}, generated by hepatic lysis of insulin-I^{131}, were being diluted by their nonradioactive analogs. Under these circumstances, the iodide generated by the action of hepatic deiodinase on iodotyrosine would be expected to be of low specific activity. These results argue against significant deiodination of intact iodoinsulin.

DISCUSSION

A postulated sequence of iodoinsulin degradation by the intact rat liver is presented in figure 8. The main purpose of this illustration is to underscore several questions concerning the interaction of insulin and intact cells. With regard to the perfusion experiments

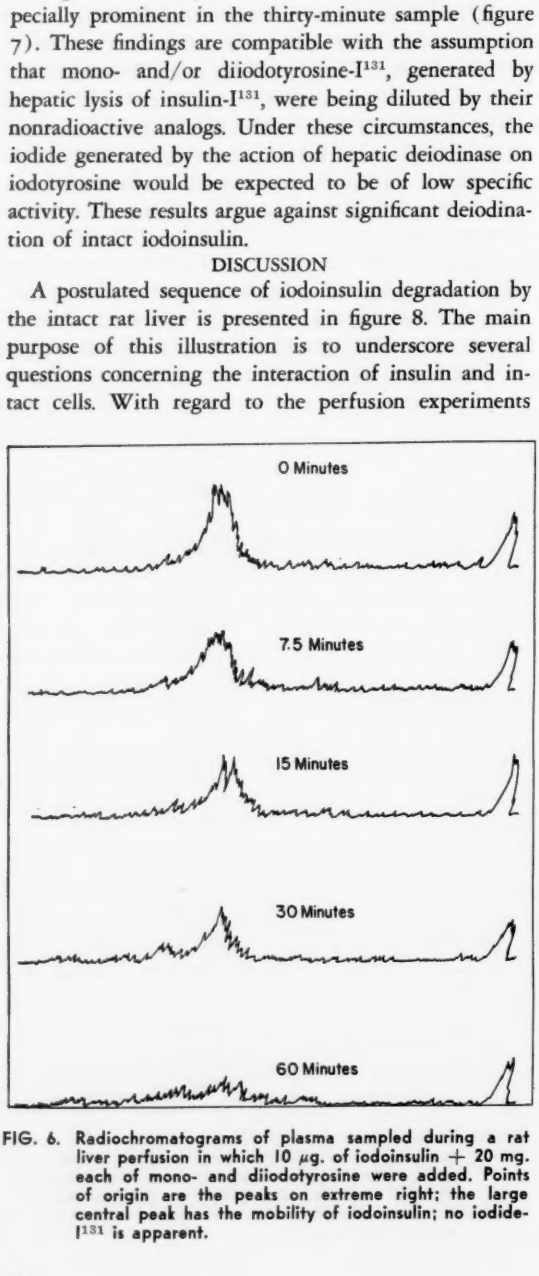

FIG. 6. Radiochromatograms of plasma sampled during a rat liver perfusion in which 10 μg. of iodoinsulin + 20 mg. each of mono- and diiodotyrosine were added. Points of origin are the peaks on extreme right; the large central peak has the mobility of iodoinsulin; no iodide-I^{131} is apparent.

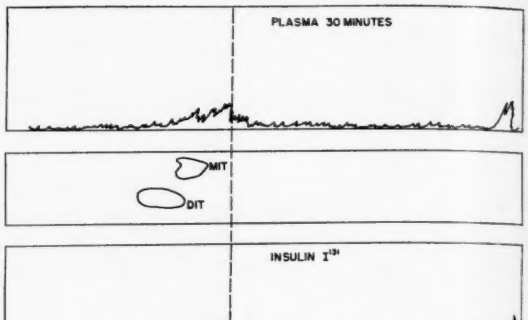

FIG. 7. Radiochromatogram of the thirty-minute plasma sample from the experiment in figure 6. Here the relative mobilities of MIT, DIT, iodoinsulin and iodide-I^{131} are shown for comparison. A peak to the left of iodoinsulin is apparently MIT. The origins are indicated by the peaks on the right.

one may ask whether or not insulin is captured and degraded by the intact cell in a manner different from the simple analogy of passive membrane diffusion and subsequent attack by soluble intracellular enzymes. Of interest in this connection is a report by Lee and Williams[20] in which the hepatic subcellular distribution of radioactivity was measured at different times following the injection of iodoinsulin into rats. Radioactivity was found to be rapidly incorporated within microsomal, mitochondrial and residual (largely soluble) fractions of the homogenized liver cell, giving a pattern of distribution which was significantly different from that obtained after the in vitro addition of either iodide-I^{131} or insulin-I^{131}. It was concluded that the liver cell may handle iodoinsulin in a way peculiar to its intact organization. The acceptance of such a view is tempting since it would prove useful in explaining the rapid uptake of insulin observed here as it has been in the interpretation of insulin effects on the isolated rat diaphragm[21] and on the enhancement of hexose penetration into eviscerated dog tissues.[22]

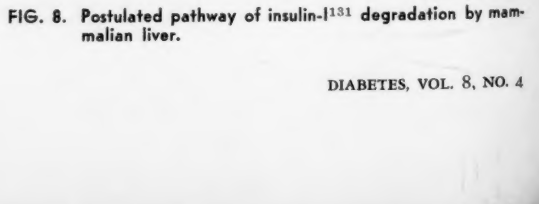

FIG. 8. Postulated pathway of insulin-I^{131} degradation by mammalian liver.

The nature of the intermediate steps before iodotyrosine release is equally obscure. Letters A and B (figure 8) indicate the possible occurrence of more than one reaction and thus more than one intermediate peptide product formed. Such reasoning is in keeping with the patterns of enzymatic protein hydrolysis observed in other situations. Although it seems certain from the experiments reported in this paper that the release of iodide requires the preliminary hydrolytic liberation of iodotyrosine, the full extent of insulin degradation is not known. Whether or not TCA-precipitable fragments are released from intact liver during insulin breakdown is a question currently under investigation.

The use of iodinated proteins as tracers in biological systems poses a problem of homogeneity, or lack thereof, within the substrate. It is known that the heavy iodination of insulin is associated with the nearly total loss of biological activity,[23,24] some of which may be restored by its partial deiodination.[28] In view of the reported greater ease in iodinating monoiodotyrosine than tyrosine,[25] it is conceivable that a substantial portion of the iodine may be added to a relatively few insulin molecules.* Under such conditions one might expect a significant fraction of the label to exist in biologically inactive insulin. This fraction, being small by weight, would not necessarily be detected in estimates of biological potency of the insulin sample as a whole. In addition to iodination per se the ionizing effects of gamma irradiation may give rise to further molecular alterations.[26,27]

As mentioned in the earlier kinetic discussion, a possible explanation for the apparently incomplete degradation of our iodoinsulin is the existence of an altered, nonreactive contaminant. Using a hydrodynamic flow paper electrophoresis technic Berson and associates[12] observed that from 2 to 25 per cent of the radioactivity from different preparations of lightly iodinated insulin migrated with serum proteins, leaving the larger fraction bound to paper at the origin. Although the paper-bound material disappeared from plasma logarithmically with time following the injection of iodoinsulin into rabbits, the fraction migrating with proteins remained relatively stable. These observations were confirmed by Scott and associates[28] who in addition were able to correlate the rate of disappearance of the paper-bound fraction with the disappearance of plasma insulin activity when iodoinsulin plus native insulin were administered to rabbits. Close quantitative agreement between the "apparent" inactive fraction of 29 per cent observed in our studies and those of Berson and Scott suggests that these fractions may be similar in constitution.

*The authors are presently investigating the chromatographic distribution of radioactivity in hydrolysates of iodoinsulin. The results to date, with four preparations similar to those reported herein, indicate that from 26 to 29 per cent of organically-bound radioactivity was incorporated in DIT; the remainder was in MIT.

SUMMARY AND CONCLUSIONS

1. The degradation of insulin-I^{131} by isolated, cyclically perfused rat livers was investigated by following with time the changing distribution of radioactivity among the plasma TCA-soluble, plasma TCA-precipitable and cellular fractions of the whole blood perfusates.

2. At relatively low concentrations of insulin-I^{131} the rate constant for the disappearance of plasma TCA-precipitable radioactivity was about 3 ml./min. Thus, at a hepatic flow rate of 7.0 ml./min., approximately 40 per cent of the insulin-I^{131} presented to the isolated, perfused liver was removed during any single passage.

3. The rate of degradation of insulin-I^{131} by perfused hind-limb preparations was far less than that of similarly perfused livers.

4. The administration of tolbutamide (Orinase) at a level of 225 mg. per kg. body weight to both blood- and liver-donor rats three hours prior to perfusion did not affect the rate of degradation of insulin-I^{131}.

5. A progressive decrease in the rate of degradation of insulin-I^{131} concomitant with increasing dilution by native insulin was observed and is indicative of substrate competition for one or more rate limiting reactive steps. Such evidence is compatible with the thesis that the degradative pathway for insulin-I^{131} may be shared in part or in its entirety by native insulin.

6. The addition of unlabeled mono- and diiodotyrosine to perfusing blood abolished the chromatographic appearance of iodide-I^{131} without affecting the hepatic uptake of insulin-I^{131}. Moreover, a small peak having the mobility of monoiodotyrosine-I^{131} was recognized. Such evidence strongly suggests that "deiodination" is not an initial event, but necessarily follows the hydrolytic cleavage of insulin-I^{131} to yield iodinated tyrosine.

SUMMARIO IN INTERLINGUA

Studios Del Metabolismo De Insulina A I^{131}

1. Le degradation de insulina a I^{131} per isolate, cyclicamente perfusionate hepates de ratto esseva investigate per sequer—como function del tempore—le alterationes in le distribution de radioactivitate in tres fractiones del perfusionato de sanguine integre, i.e. le fraction de plasma solubile in acido trichloroacetic, le fraction de plasma precipitabile per acido trichloroacetic, e le fraction cellular.

2. A relativemente basse concentrationes de insulina a I^{131}, le magnitude constante del disparition de radioactivitate in le fraction precipitabile per acido trichloro-

acetic esseva circa 3 ml per minuta. Assi, in le presentia de un fluxo hepatic de 7,0 ml per minuta, approximativemente 40 pro cento del insulina a I^{131} presentate al isolate e perfusionate hepate de conilio esseva eliminate durante un sol passage.

3. Le intensitate del degradation de insulin a I^{131} per perfusionate preparatos de gamba posterior esseva multo inferior a illo constatate in le caso de similemente perfusionate hepates.

4. Le administration de tolbutamido (Orinase) in un dosage de 225 mg per kilogramma de peso corporee al rattos contribuente le sanguine e etiam al rattos contribuente le hepates tres horas ante le perfusion non afficeva le intensitate del degradation de insulina a I^{131}.

5. Un progressive relentation del degradation de insulina a I^{131}, concomitante con un dilution progressive per insulina native, esseva notate e pare indicar le competition del substrato pro un o plure reactiones relentatori. Tal observationes es compatibile con le these que le degradation de insulina a I^{131} passa per un sequentia de passos que es partialmente o completemente identic con illo de insulina native.

6. Le addition de non-marcate monoiodotyrosina e diiodotyrosina al sanguine de perfusion aboliva le apparition chromatographic de iodo a I^{131} sin afficer le acceptation de insulina a I^{131} per le hepate. In plus, un micre picco con le mobilitate de monoiodotyrosina a I^{131} esseva recognoscite. Tal observationes es un forte suggestion que le "disiodation" non es un evento initial sed seque le fission hydrolytic de insulina a I^{131} que resulta in le formation de tyrosina iodate.

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Effects of Insulin with and without Tolbutamide in a Depancreatized Woman

E. Perry McCullagh, M.D., and Herbert W. Goebert, Jr., M.D., Cleveland

In 1942 Loubatières hypothesized that the pancreas was necessary for the hypoglycemic action of oral sulfonamides.[1] Since then extensive experimentation has produced some, but not complete, information regarding the mechanism of the action of these drugs. The opinion was voiced recently in an editorial[2] in DIABETES that the hypothesis that the hypoglycemic action of these drugs depends upon the stimulation of the pancreatic beta cells is gaining credence.

A few studies with depancreatized human beings have substantiated Loubatières' theory. All of the patients studied were chronically or acutely ill. Those with pancreatectomies or chronic pancreatitis failed to show hypoglycemic responses to sulfonamides administered orally or intravenously.[3-6]

Following a total pancreatectomy five years ago our patient is a healthy, active woman, on whom we measured the effects of exogenous insulin on blood sugar values with and without the accompanying intravenous administration of tolbutamide.

CASE REPORT

The patient first was examined at the age of thirty-three years because of moderately severe, painless jaundice; the jaundice apparently had been present throughout a pregnancy, which had ended prematurely five months before. She weighed 104 lb.

At operation a carcinoid that involved the head of the pancreas was found. Two adjacent lymph nodes were involved. A radical pancreaticoduodenostomy and a partial gastrectomy were done, followed by a choledochojejunostomy and a gastroenterostomy. The involved lymph glands were removed. The operation was performed in January 1954.

Before surgery the laboratory findings were as follows: fasting blood sugar 94 mg. per 100 ml., blood urea 27 mg. per 100 ml., total plasma protein 6.9 gm. per 100 ml., serum albumin 3.8 gm. per 100 ml., serum amylase 930 units, and serum bilirubin (direct) 0.39 mg. per 100 ml. One day postoperatively the fasting blood sugar content was 666 mg. per 100 ml. Regular insulin was prescribed: 4 units three times after meals the first day, and 6 units four times the second day. Two weeks postoperatively she was taking 16 units of NPH insulin before breakfast; the blood sugar content was 97 mg. per 100 ml. before the evening meal, and 131 mg. per 100 ml. after fasting.

The diabetes was labile. Diarrhea was difficult to control. Seven months postoperatively a pelvic abscess required drainage. Anxiety and moderately severe depression were other complications. Fourteen months postoperatively the patient's weight had declined to 94 lb.

At present, five years postoperatively, she is in excellent health. She weighs 121 lb. She has moderate arterial hypertension, with blood pressure at 170/110 mm. Hg. Retinopathy is not present. The diarrhea has stopped. Her diet consists of carbohydrate 215 gm., protein 72 gm., and fat 67 gm., in six feedings per day. The NPH insulin dose had been increased to 24 units per day. Blood sugar levels two days before the test to be described were 213 mg., 176 mg., and 141 mg. per 100 ml. before the morning, noon and evening meals, respectively. Urine tests done on single specimens before meals showed three-plus glycosuria before breakfast, four-plus before lunch, and were negative before the evening meal and at bedtime. The serum calcium content was 9.4 mg. per 100 ml., and the serum phosphorus content was 4.3 mg. per 100 ml. No 5-hydroxyindoleacetic acid was found in the urine.

PROCEDURE

Two tests were done on separate days. In the first test tolbutamide was not given, and in the second test the drug was administered.

On the first day, 1,000 ml. of a 5 per cent solution of dextrose in water was given intravenously over a four-hour period in the morning to the fasting patient. This amount of dextrose was approximately equivalent to the available glucose in her usual breakfast. Six units of regular insulin was added to the solution because this amount was approximately that required to control the blood sugar content after the ingestion of an equivalent amount of glucose. Blood was drawn for blood glucose determinations before beginning the intravenous administration of dextrose and insulin, hourly for four hours during its administration, and one hour after absorption; the graphed curve is shown in figure 1. The patient ate her regular diet at the noon meal and for the remainder of the day. Twenty units of NPH insulin was given after the intravenous solution was absorbed.

On the second day the patient's usual diet and insulin dosage were resumed. On the third day, one and one-half hours before beginning the administration of intravenous fluid, blood was drawn for a determination of the blood sugar content, and 2 gm. of tolbutamide was given intravenously. The second dose of 2 gm. of tolbutamide was given when the

From the Department of Endocrinology, The Cleveland Clinic Foundation, and The Frank E. Bunts Educational Institute, Cleveland, Ohio.

TABLE 1

Blood glucose values (in a woman who had undergone pancreatectomy) in tests with and without tolbutamide

Intravenous infusion of 1,000 ml. 5 per cent dextrose in water and six units regular insulin	Blood glucose determinations, mg./100 ml.						
	Before intravenous infusion		During 4-hr. infusion (hourly)				After infusion
	1½ hr. before	Immediately before	1 hr.	2 hr.	3 hr.	4 hr.	1 hr.
Without tolbutamide		179	250	250	214	208	171
With intravenous injection of tolbutamide	142 (2 gm. tolbutamide)	155 (2 gm. tolbutamide)	241	285	227	208	161

intravenous administration of 1,000 ml. of water containing 5 per cent dextrose and 6 units of regular insulin was begun. The intravenous infusion was given over a period of four hours. Two hours after it was begun, blood was drawn for a determination of the tolbutamide content of the blood;[7] the findings were checked against a blank and later against the patient's blood. The blood glucose concentrations throughout the test are shown in figure 1. The values represented in figure 1 are listed in table 1. The determinations of blood sugar were done by the Somogyi-Nelson method, in duplicate, with great care by an expert technician.

It is seen from figure 1 and from table 1 that the blood glucose concentrations in the first two tests on the day of the administration of tolbutamide were somewhat lower than the concentration before the test in which no tolbutamide was given. It seems unlikely that tolbutamide was responsible in view of the subsequent figures. The blood glucose levels were not, however, within the normal range. The remaining portions of the curves are remarkably similar, especially in view of the fact that the diabetes was so unstable. The blood sugar content rose slightly higher following the administration of tolbutamide than it did during the test on the preceding day. It was slightly—but not significantly—lower after five hours.

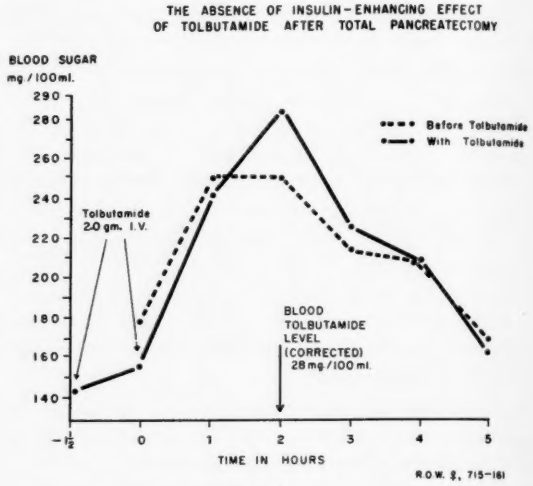

FIGURE 1

CONCLUSIONS

We were unable to demonstrate in a healthy young woman who had undergone total pancreatectomy that the hypoglycemic effect of insulin after the administration of tolbutamide was greater than the hypoglycemic effect of insulin in the absence of tolbutamide.

SUMMARIO IN INTERLINGUA

Le Effectos De Insulina, Con E Sin Tolbutamido, In Un Dispancreatisate Patiente Feminin

Nos non poteva demonstrar, in un juvene femina de bon stato de sanitate qui habeva essite subjicite a pancreatectomia total, que le effecto hypoglycemic de insulina post le administration de tolbutamido esseva plus grande que le effecto hypoglycemic de insulina sin le administration de tolbutamido.

ACKNOWLEDGMENT

We wish to thank Miss Victoria Asadorian for her technical assistance.

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EDITORIAL

THE SIGNIFICANCE OF THE ACTION OF INSULIN UPON MUSCLE MEMBRANE POTENTIAL

Two observations placed in juxtaposition may clarify the mechanism of action of insulin. The first is that insulin in contact with intact rat muscle causes net movement of potassium from extracellular fluid into muscle. The second is concerned with the electrical potential difference across the cell membrane of resting muscle which depends in part upon the relative concentrations of potassium on either side of the membrane. Let us consider these observations, beginning with the second.

Using an appropriate apparatus, it can be shown that there is a potential difference between electrodes placed inside and outside a muscle fiber. This means that the membrane is polarized. The inside of the fiber is negative. The potential varies in different preparations. In excised frog muscle[1] and in leg muscles in situ in adult rats,[2] the resting membrane potential, E_R, is about —90 mV. In excised leg muscles of immature rats E_R is generally between —70 and —75 mV.[3]

Nernst developed a relation between the ion concentrations of two conjoined solutions of a common electrolyte and the electrical potential difference between them. Later Donnan arrived at an expression identical to Nernst's. In the system used by Donnan, the presence of a nondiffusible ion displaces diffusible ions so that at equilibrium the concentrations of each species of diffusible ion on the two sides of the membrane are unequal. As a result of inequality of ion concentration there must be a potential difference across the membrane. The size of the potential is predicted by the product of a temperature-dependent constant and the logarithm of the ratio of the concentration of any one of the diffusible ions on one side of the membrane to its concentration on the other side, viz.

$$E = \text{constant} \times \log \frac{I_1}{I_0}$$

Höber[4] and Bernstein[5] were probably the first to apply these proposals from physical chemistry to examination of potentials in living systems. They reasoned that the high concentration of potassium inside cells, compared to its low concentration outside, might account for the observed high injury potentials.

After so fruitful a beginning the matter rested for several decades owing to lack of refined technics for measuring potentials. Around 1940 Cole and Curtis[6] made precise measurements by impaling the giant axon of the squid with an electrode. Interest in the relation between ion concentrations and bio-electrical phenomena was revived.

Boyle and Conway[7] predicted from Donnan's equations that the product of potassium and chloride concentrations in muscle-fiber water should equal the same product in extracellular water. From their measurements in frog muscle they concluded that these substances did indeed behave as though muscle were a Donnan membrane system. It might be hoped, then, that E_R could be explained entirely by the Donnan phenomenon.

So simple an explanation, however, is not quite tenable. Both the Nernst and the Donnan treatments of simple solution systems assume equilibrium conditions. E_R is not completely described either by the predicted equilibrium potassium potential or by the predicted equilibrium chloride potential. The calculated potassium equilibrium potential in frog and in rat muscle is about —100 mV, a value uniformly higher than the observed value, E_R.

Despite the complexities of interpreting E_R quantitatively in terms of ion concentrations when these are normal, there is no doubt that when extracellular potassium concentration is raised enormously, say by tenfold or more, E_R in excised nerve and muscle can then be described fairly accurately by the product of a constant and the logarithm of the ratio of intracellular to extracellular potassium concentrations.[1,6] Either an increase in extracellular potassium concentration or a decrease in intracellular potassium concentration decreases the ratio of concentrations and decreases the membrane potential; the membrane is hypo- or depolarized. An increase in intracellular or a decrease in extracellular potassium concentration increases the ratio of concentrations and increases the membrane potential; the membrane is hyperpolarized.

With this background, we can return to the second observation: Insulin produces a net movement of potassium into muscle. In the intact animal in which intracellular potassium mass is very large compared to extracellular potassium mass, net movement of potassium into cells causes only a minute fractional increase in intracellular potassium though it may reduce extra-

cellular potassium considerably. For example, if half the extracellular potassium in man suddenly shifted into muscle, the concentration of potassium in muscle would increase by only about 1 per cent. Such movement of potassium should lead to hyperpolarization of muscle, for reasons discussed in the preceding paragraph.

However, before we accept the possibility that insulin-induced potassium shifts might hyperpolarize muscle, let us ask why potassium moves into muscle under the influence of insulin. It is generally assumed that it does so because insulin has a primary effect upon glucose uptake. This view, possibly correct, is not yet supported by substantial data and is opposed by observations suggesting independence of potassium uptake from increased glucose uptake due to insulin action.

With these reservations in mind we can re-examine the statements made previously about the relation between membrane potential and ion ratios. The equations can be inverted. If it is true that E_R depends on ion ratios, it is equally true that if by some independent means we can fix E_R and maintain it at its new value, then ions must flow from one side of the membrane to the other until their ratio conforms to that demanded by the new E_R.

These ideas have been explored in part. When insulin is added to a solution bathing rat muscle the membrane potential is increased.[8] In this experiment, in which extracellular potassium concentration is held constant, insulin effect on potassium movement is demonstrated only by an increase in intracellular potassium concentration. The question is which is the horse and which the cart. Serial measurements of potassium concentration in muscle compared to measurements of E_R show that intracellular potassium increases only slowly in the presence of insulin. After three hours' exposure to insulin intracellular potassium is still not as high as predicted by the increase in membrane potential produced in only one hour's exposure to insulin; that is, insulin probably hyperpolarizes muscle before there is appreciable movement of potassium. The degree of hyperpolarization is at least adequate to account for the subsequent movement of potassium. If this is a correct description of the sequence of events, the next question is by what means does insulin produce hyperpolarization. Whatever the intimate details of the answer, the observation hints strongly that insulin acts directly on the muscle membrane. This conclusion, when taken in conjunction with other evidence in the literature, lends added weight to the validity of the transport hypothesis as a prime action of insulin in accelerating glucose metabolism by muscle.

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KENNETH L. ZIERLER, M.D., Baltimore

BOOK REVIEWS

PSYCHOLOGICAL ASPECTS OF AGING. *Edited by John E. Anderson. $2.00, pp. 323, American Psychological Association, Washington, D.C., 1956.*

The "Conference on Planning Research" is one of the current scientific fashions. Whether such conferences succeed in stimulating research is not clear, but occasionally the papers presented at them can be stimulating. This volume represents the proceedings of such a conference on "Planning Research on the Psychological Aspects of Aging." It contains more than a score of papers on personal and social adjustment; on the assessment of aging; on perceptive and intellective abilities; on learning, motivation and education; and on functional efficiency, skills and employment—contributed by social and behavioral scientists from universities throughout the country.

Although the volume contains little that the practitioner can apply directly to the treatment of his patients, and little that the investigator can utilize immediately, it contains a great deal of food for thought. For example, William E. Henry's paper on "Affective Complexity and Role Perceptions," despite its formidable title, considers some very important problems about men and their relation to the world around them. John B. Calhoun's paper on "The Use of Animals in Research on Aging" reminds us again that aging is a phenomenon of all metazoan organisms, and that much can be learned from the laboratory animal. Nancy Bayley and Wayne Dennis have each contributed papers indicating how much can be learned from the longitudinal study of the patient. Ross A. McFarland in his description of the functional efficiency, skills and employment of the aged illustrates how much more precisely one can measure the physiological changes which come with age than the psychological.

Since the management of diabetes is always of long duration, and since a patient with diabetes is most frequently in the older age group, those who concern themselves with the design of therapeutic or investigative procedures to be used with diabetics will do well to study this volume.

THE EARLY DETECTION AND PREVENTION OF DISEASE. *Edited by John P. Hubbard, M.D. $7.50, pp. 350, McGraw-Hill Book Company, Inc., New York, 1957.*

This book is a collection of a five-day symposium arranged and directed by the Department of Public Health and Preventative Medicine of the University of Pennsylvania School of Medicine, in cooperation with the American College of Physicians.

It outlines very clearly the various problems associated with preventative medicine in clinical practice. The various sections on cardiovascular disease and the gastrointestinal system are well presented and demonstrate that clinicians do think of specific diseases from their preventative as well as diagnostic and clinical viewpoints.

This reviewer was impressed with the section by Dr. Lemuel C. McGhee entitled "The Demand for the Clinical Practice of Preventative Medicine." It proved both stimulating and enlightening. Likewise, the section regarding periodic health examinations outlines clearly the importance and rewards of such examinations.

This book will be both interesting and useful to the clinician and student, and should help correlate and orient us all in this important phase of medical practice.

MANUAL OF CHEST CLINIC PRACTICE IN TROPICAL AND SUBTROPICAL COUNTRIES. *By A. J. Benatt, M.D. $3.00, pp. 100, E. S. Livingstone Ltd., Edinburgh and London, 1959. The Williams & Wilkins Co., Baltimore, exclusive U.S. agents.*

This volume is small in size but large in contents. Its primary purpose is to serve as a manual for the conduct of a tuberculosis clinic in tropical and subtropical countries. It was written by the late Dr. Benatt to help others who may have to treat tuberculosis under difficulties and is based on his experiences with such problems in projects under the auspices of the World Health Organization. Hence its emphasis on the practical and socio-economic aspects of the care of this disease in the less developed countries. Of particular interest to the readers of DIABETES is the chapter on the management of the tuberculous diabetic. In a few clearly written pages a concise, accurate, and eminently practical guide is presented for the therapy of this most serious combination of diseases.

The author has fulfilled his purpose admirably. One can read between the lines of this utilitarian volume and sense the deep humanitarianism of the author. He has created a vade mecum which should be of great assistance in the field.

TRAITEMENT DU DIABETE INFANTILE EN REGIME LIBRE. *By P. Royer and H. Lestradet. 3,500 francs ($7.18), pp. 194, Editions Médicales Flammarion, Paris, 1958.*

This well-written, well-organized monograph ably supports the thesis that diabetic children can be maintained in good health, with normal growth and development, without rigid rules for a strictly prescribed diet, measured or weighed. The booklet of 190 pages summarizes the authors' experience with some 800 diabetic children whose care was directed under the regimen commonly called "free diet" (régime libre) in the Hôpital des Enfants Malades at Paris. In historical perspective the authors cite the proposals and arguments supporting this regimen that have been developed during more than thirty years, principally by Stolte in Germany, Lichtenstein and Larsson in Sweden, Fanconi in Switzerland, Payne in England, and by Tolstoi and Guest in the United States. They present judiciously the controversial opinions regarding different dietary rules for diabetic management expressed by proponents and opponents of the different regimens. (See a similar discussion in two editorials pro and con, published in DIABETES 1:487, 1951).

In the preface, Professor Julien Marie states sensibly that practical experience has convinced many pediatricians that regimens restricting carbohydrates for diabetic children were not useful; more than that, restrictive diets actually led often to dystrophic manifestations that were at first mistakenly thought to be part of the evolution of juvenile diabetes, e.g., dwarfism and hepatomegaly, the "Mauriac Syndrome." Such manifestations are now more accurately ascribable to poorly regulated insulin dosage in relation to food intake—regardless of the quality of dietary planning (see editorial in DIABETES 2:415, 1953).

Successive sections of the book offer concise discussions of the physiopathology of juvenile diabetes, its diagnosis and the differential diagnosis of nondiabetic meliturias, different schemes of treatment, different types of insulin and reasons for the choice of one or another type in individual cases, technic of insulin injection and equipment needed. Practical considerations are emphasized in the discussion of the most essential features of instructions given to parents and to the children for guidance of their daily self-management. The chapter on urine-testing is especially clear, offering concise descriptions of technics and of the commercially available simple reagents for testing for sugar and for acetone. The paragraph that ends this chapter merits quotation here, freely translated: "It has been said that repeated frequent testing of urine creates an intolerable (i.e., psychologically unhealthy) attitude of anxiety in the family. We have not had this experience when all the interested members of the family are made to understand the objectives of the testing and to appreciate the feeling of security derived from the results of the tests. This daily practice, far from unduly fixing the thoughts on the malady, affords a feeling of liberation from worries when adaptation of daily insulin dosage to changing physiological needs is successful, because, if successful, the development of acidosis and coma should never occur and the occurrence of hypoglycemic reactions should be rare."

An extensive section of the book is devoted to the physiopathology and treatment of complications, acute and chronic, concomitant endocrinologic factors and nutritional disturbances. Final chapters deal with medico-social problems, schooling, centers for education, vacation camps, and lay associations in France (Association Française des Diabétiques and the Association pour l'Aide aux jeunes Diabétiques).

ABSTRACTS

Altszuler, N.; Steele, R.; Wall, J. S.; Dunn, A.; and de Bodo, R. C. (Dept. of Pharmacol., New York University Coll. of Med., New York City, and Dept. of Biology, Brookhaven National Lab., Upton, N. Y.): EFFECT OF GROWTH HORMONE ON CARBOHYDRATE METABOLISM IN NORMAL AND HYPOPHYSECTOMIZED DOGS: STUDIES WITH C^{14} GLUCOSE. Am. J. Physiol. *196*:121-24, January 1959.

Using trace amounts of C^{14} glucose, introduced intravenously by a priming injection along with a continuous constant infusion, the effect of a growth hormone regimen on the size of body glucose pool and on the rate of glucose utilization as well as production was studied in unanesthetized normal and hypophysectomized dogs. In both types of animals the growth hormone regimen increased the size of the body glucose pool. More significantly the growth hormone regimen increased the rate of glucose utilization as well as the rate of glucose production. These effects occurred in adrenalectomized animals, indicating that growth hormone can exert profound effects in the absence of the adrenal gland. The source of the increased glucose produced during the growth hormone regimen is not known but conceivably results from glucose spared and released into the plasma in consequence of increased hepatic fat metabolism brought about by the growth hormone.

It is proposed that the inhibitory effect of injected growth hormone on glucose uptake observed in isolated diaphragm is overcome in vivo by an increased endogenous insulin secretion evoked by the extra glucose produced as a result of the growth hormone treatment. This concept of an enhanced glucose uptake because of secondary stimulation of increased insulin output does not account for the immediate increase in glucose utilization and hypoglycemia observed under special conditions in certain species including depancreatized animals. L.S.S.

Argyris, Bertie F. (Zoology Dept., Syracuse University, Syracuse, N. Y.): THE MECHANISM OF INSULIN RESISTANCE IN THE KL STRAIN OF MICE: INACTIVATION OF INSULIN. Endocrinology *64*:400-08, March 1959.

The KL strain of mice is able to tolerate the injection of a large amount of insulin (300-500 U.) without exhibiting convulsions. This resistance to insulin cannot be explained by an increased rate of insulin destruction. An expected prompt fall in blood sugar is seen after the injection of large amounts of insulin into KL mice but the decline halts at about 30 mg. per cent. The blood sugar of control mice continues to fall below 30 mg. per cent and convulsions ensue. The resistance of KL mice to insulin-induced convulsions seems to reside in their ability to maintain blood sugars just above the convulsive level. R.L.J.

Bryan, George T.; Read, Charles H.; and Zimmerman, George R. (State University of Iowa Coll. of Med., Iowa City, Ia.): DISSEMINATED MUCORMYCOSIS IN A CHILD WITH DIABETES MELLITUS. J. Iowa M. Soc. *48*:193-96, April 1958.

The clinical features and necropsy findings of a case of disseminated mucormycosis in a thirty-four-month-old child with diabetes mellitus have been described. The diagnosis of mucormycosis was established on the basis of the characteristic thrombotic lesions and on the histologic evidence that these are due to a fungus of the family Mucoraceae. Pertinent literature has been briefly reviewed, and allusion has been made to possibly significant host-parasite relationships. W.R.K.

Cantor, Edward B.; Silverman, Melvin; Baladi, Kamel; and Brahen, Louis J. (Depts. of Obst. and Pediat. and Div. of Labs., Cedars of Lebanon Hosp., Los Angeles, Calif.): HYALINE MEMBRANE DISEASE. Obst. & Gynec. *12*:632-41, December 1958.

The authors have reviewed thirty-six cases of neonatal deaths due to hyaline membrane disease. They discussed theories of etiology and predisposing factors and possible methods of prevention as well as active therapeutic measures. E.A.W.

Cavallero, Cesare (Letters to Editor). (Inst. of Pathological Anat., University of Pavia, Italy): GLUCAGON AND BODY GROWTH IN DWARF MICE. Lancet *1*:521, March 7, 1959.

The author refers to a previous paper in which he concluded that pancreatic glucagon can stimulate body growth in pituitary dwarf mice. His present letter offers further data to support this conclusion. C.A.R.

Chernick, Sidney S.; and Scow, Robert O. (Lab. of Nutrition and Endocrinology, National Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.): EARLY EFFECTS OF "TOTAL" PANCREATECTOMY ON FAT METABOLISM IN THE RAT. Am. J. Physiol. *196*:125-31, January 1959.

Within two hours after "total" pancreatectomy in fasting rats blood concentrations of glucose, ketone bodies and total lipids were significantly elevated. Blood ketone bodies and total lipids increased before the blood glucose reached diabetic levels. The increase in blood lipids was due largely to triglycerides and was accompanied by accumulation of fat in the liver and kidneys. The total carbohydrate content of the liver of depancreatized rats eighteen hours after surgery was three times greater than that of controls. Ketosis developed in these animals despite an hepatic carbohydrate content of almost 1 per cent. When depleted of body fat prior to surgery, pancreatectomized rats did not develop ketosis, hyperlipemia or fatty liver and kidneys.

Repeated injections of insulin started immediately after pancreatectomy prevented development of diabetes. When insulin was given for the first time one day after pancreatectomy, blood glucose, ketone bodies and fats were lowered to normal fasting levels within three hours; two days after pancreatectomy, blood glucose and fats were reduced to normal levels whereas the ketonemia, although somewhat decreased, remained high. The rapid rise in blood lipids and ketone bodies following pancreatectomy in the rat, starting even before the blood glucose level had increased to the diabetic range above 150 mg./100 cc., and the prompt response of these indices to insulin administration are interpreted as evidence that insulin has a direct effect on these phases of fat metabolism. The decreased insulin effect on ketonemia in rats deprived of insulin for two days may be related to the increased hepatic fat content forty-eight hours after pancreatectomy and the close correlation between the degree of ketosis and the amount of fat in the liver. L.S.S.

Chimènes, Hubert (Correspondence). (Paris, France): UNSTABLE DIABETICS AND LIPODYSTROPHY. Brit. M. J. *1*:170, Jan. 17, 1959.

The author reports the case histories of two patients with insulin lipodystrophies and unstable diabetes. He is convinced that the vast majority of unstable, "brittle" diabetics are wholly due to insulin lipodystrophies, unobserved by the doctors and neglected by the patients. C.A.R.

Chulski, Thomas (Dept. of Product Research & Development, The Upjohn Co., Kalamazoo, Mich.): SPECTROPHOTOMETRIC DETERMINATION OF TOLBUTAMIDE IN PLASMA. J. Lab. & Clin. Med. 53:490-94, March 1959.

The author presents a technic for the spectrophotometric determination of tolbutamide in plasma. G.J.H.

Colwell, Arthur R., Jr.; and Colwell, John A. (Dept. of Med., Northwestern Univ. Med. School, Chicago, Ill.): PANCREATIC ACTION OF THE SULFONYLUREAS. J. Lab. & Clin. Med. 53: 376-95, March 1959.

The site of the action of the sulfonylureas was investigated by injecting solutions of the compounds into the blood vessels supplying the pancreas and liver. Perfusion of the pancreas via its arterial circulation with small amounts of tolbutamide in the anesthetized dog caused moderate hypoglycemia, while little or no such effect was obtained when the femoral vein or hepatic artery was perfused with the same amount of drug. There was also no hypoglycemic response following injection of carbutamide into the portal vein. There was no alteration of morphology of alpha or beta cells following perfusion of the main pancreatic artery with carbutamide. A major contribution of this paper is a fairly thorough review of pertinent literature describing the mechanism of action of the sulfonylureas. The authors' conclusion is that the primary action of sulfonylurea compounds is by their effect on the pancreatic islands. G.J.H.

Cosnett, J. E. (King Edward VIII Hosp., Durban, Union of South Africa): DIABETES AMONG NATAL INDIANS. Brit. M. J. 1:187-92, Jan. 24, 1959.

The author reports that diabetes mellitus is exceptionally common among Natal Indians. An analysis of 207 diabetics revealed certain peculiarities, some of which were previously described in other tropical and subtropical countries. The typical labile, insulin-sensitive type of diabetes in young people is uncommon. Instead, a form of juvenile diabetes occurs which is characterized by insulin resistance and freedom from ketosis. Between ages twenty and forty there is a group of overweight diabetics, mostly women, who are more sensitive to insulin. Beyond forty years of age, insulin requirements varied, but patients are more often underweight than overweight. Ketosis is rare among Natal Indians, but infections and vascular complications of diabetes are common. There is a resemblance in several respects between the diabetic population herein described and that noted in the island of Jamaica. C.A.R.

Dodds, Sir Charles; Fairweather, F. A.; Miller, A. L.; and Rose, C. F. M. (Courtauld Inst. of Biochemistry, Middlesex Hosp., London, W.1, England): BLOOD-SUGAR RESPONSE OF NORMAL ADULTS TO DEXTROSE, SUCROSE, AND LIQUID GLUCOSE. Lancet 1:485-88, March 7, 1959.

The authors report the blood sugar response to amounts of dextrose, sucrose, and liquid glucose equivalent to 45.4 gm. of carbohydrate in nineteen subjects. All these carbohydrates were rapidly absorbed and produced a rapid rise in blood sugar to a maximum which usually was reached within thirty minutes. Reference is made to diagnostic glucose-tolerance tests, with the warning that blood sugar peaks may be missed if timing of the blood samples does not take into account the above observation. Glucose in solution produced a more rapid rise than sucrose. The rise in blood sugar was predominantly due to glucose no matter which carbohydrate was administered. There was some evidence, as yet unsubstantiated, that the amount of nonglucose reducing substances in the blood increased after sucrose. C.A.R.

Dotevall, Gerhard (Med. Dept., Varberg Hosp., Varberg, Sweden): SECONDARY RESISTANCE IN ORAL TREATMENT OF DIABETES. Acta med. scandinav. 161:251-56, June 9, 1958.

Out of a group of sixty-five diabetics treated with BZ-55 (carbutamide) or D-860 (tolbutamide), fifteen patients developed secondary resistance after an observation period of from two to eighteen months. By secondary resistance, the author means patients who have primarily shown a favorable response, but have relapsed after two months' or longer period of treatment. Nine of the patients who developed secondary resistance had previously taken insulin for an average of 2.2 yr. Their mean age was 64.6 yr., and the mean duration of diabetes was 6.7 yr., compared to 2.5 yr. in the patients who did not develop secondary resistance. Constitutionally, the majority of the secondary resistance patients belonged to the pyknomorphic group. Most of them did not require a greater quantity of insulin after the discontinuation of oral treatment. Various theories of the cause of secondary resistance are discussed. The majority of the cases supported the theory of reversible collapse of the beta cells in the pancreas. It is felt that the criteria for oral treatment should be more stringent. Indications for change-over from insulin to oral therapy should be particularly stringent. Even in cases otherwise suited to tablet therapy, dieting should first be attempted and adipose patients should be put on a weight-reducing regimen. Oral therapy should be used only if dieting proves unsuccessful. If secondary resistance develops the patient should be transferred to insulin. B.F.K.

Dyrbye, M. O. (The Connective Tissue Res. Lab., Univ. Inst. of Med. Anat., Copenhagen, Denmark): STUDIES ON THE METABOLISM OF THE MUCOPOLYSACCHARIDES OF HUMAN ARTERIAL TISSUE BY MEANS OF S^{35} WITH SPECIAL REFERENCE TO CHANGES RELATED TO AGE. J. Gerontology 14:32-36, 1959.

The metabolism of the acid sulfomucopolysaccharides in human aortas was studied in vitro by means of S^{35}-labeled inorganic sulfate. Sixteen samples were incubated with the radiosulfate, the mucopolysaccharides were isloated by chemical means and the uptake of S^{35} in the isolated material was determined. A decreasing uptake with increasing age and arteriosclerosis was observed. S.S.

Editorial. DIABETES IN THE TROPICS. Brit. M. J. 1:219-20, Jan. 24, 1959.

Diabetes seems to be relatively mild among people of the tropics. Illiteracy and poverty make for poor dietary control and insulin treatment, yet ketosis and diabetic coma are rare. Reference is made to the two common types of diabetics in temperate regions who are either labile or stable and who either have total or partial insulin deficiency. Studies of diabetics in Jamaica revealed a third variety, not previously described, which was present about half as often as the usual juvenile-type cases. The third variety, labeled type "J," started in young persons or even children, who were underweight but, unlike juvenile diabetics, were insensitive to insulin and often required 100

or more U. daily for control; moreover, they did not readily develop signs of ketosis if these large insulin doses were withdrawn. There were also some examples of apparently temporary diabetes in young persons. These peculiarities in diabetics in Jamaica have been confirmed and have been noted elsewhere in the tropics, more recently among the Natal Indian population. Study of the nature of diabetes of the tropics should contribute to a better understanding of the mechanism of the diabetic syndrome. The results might well lead to a better understanding of the disorder. C.A.R.

Ellenberg, Max (Dept. of Med., Mount Sinai Hosp., New York, N. Y.): DIABETIC NEUROPATHY PRECIPITATED BY DIABETIC CONTROL WITH TOLBUTAMIDE. J.A.M.A. *169*:1755-57, April 11, 1959.

In three patients, the onset of diabetic neuropathy followed the institution of control of hyperglycemia and glycosuria with tolbutamide. The neuropathy was identical with the recognized forms of diabetic neuropathy, including symptoms, signs, and course. The neurological picture improved and symptoms disappeared despite the continued use of tolbutamide. Similar experiences have been reported with control of diabetes by insulin or dietary restrictions. Thus, tolbutamide is not the noxious agent but acts as the medium for control of the diabetes which, in certain instances, leads to neuropathy. B.F.K.

Fenton, E. L. (Wellcome Research Lab., Biological Div., Langley Court, Beckenham, Kent, England): A METHOD FOR THE ASSAY OF INSULIN BY PAPER CHROMATOGRAPHY. Biochem. J. *71*:507-13, March 1959.

The author reports that insulin can be separated from associated impurities and inactive material by ascending chromatography with the upper phase of butan-2-ol, 1 per cent acetic acid (1:1, v/v). A solution of bromocresol green buffered with sodium acetate is used for locating the insulin zones. A linear relationship exists between the amount of insulin appearing in the specific zone and the biological potency of the sample. By elution of the dye from the specific insulin spot and quantitative colorimetric comparison with the similar zone from a standard of known potency, the biological potency of the sample can be estimated. Data are recorded on the comparison between biological potency and the potency given by the chromatographic method for samples of crystalline and crude insulins and for solutions of insulin which had undergone partial decay as a result of prolonged storage under unfavorable conditions. Close agreement has been obtained between the mouse-convulsion method and the chromatographic assay. C.A.R.

Field, James B. (Clin. Endocrin. Branch, Nat. Inst. of Arthritis & Metabolic Diseases, Nat. Inst. of Health, Bethesda, Md.): STUDIES ON THE CIRCULATING INSULIN INHIBITOR FOUND IN SOME DIABETIC PATIENTS EXHIBITING CHRONIC INSULIN RESISTANCE. J. Clin. Invest. *38*:551-56, March 1959.

This is an investigation of the presence of an antagonist to the action of insulin in the blood of ten patients with chronic insulin resistance. Their insulin requirements ranged from 185 to 2,000 U. per day. Multiple technics were used for detection and measurement of insulin antagonism as follows: the effect of insulin upon glycogen deposition in rat hemidiaphragm, the protection of mice from lethal doses of insulin, insulin binding in vitro, and the serum gamma globulin content. By the first method they were able to detect insulin antagonism in two patients, although they were not the ones using the most insulin. The mouse assay method was positive in two patients who, however, showed no insulin antagonism by the first method. Insulin binding of insulin was impressively increased in all but one patient. This patient required 2,000 U. of insulin per day but was classified a "lipo-atrophic" type of diabetic. In one patient, ACTH therapy reduced both insulin requirement and antagonism (by the diaphragm method) but not the insulin binding. The diaphragm method also showed that the insulin binding effect was present for human insulin prepared by the authors themselves. The sera of all but one of the patients bound increased amounts of insulin to the gamma globulin fraction. S.B.B.

Fodden, John H. (Dept. of Pathology, University of South Dakota Sch. of Med. Sciences, Vermillion, S. D. Present Address: Div. of Labs., Mount Sinai Hosp., Milwaukee, Wisc.): THE EFFECTS OF HORMONES UPON HEPATIC GLYCOGENESIS. A.M.A. Arch. Path. *66*:10-22, July 1958.

A variety of experimental circumstances has been used to examine the hepatic function of glycogenesis. One of its objects was to determine or clarify relationships between the actions of insulin and adrenal glucosteroids within this physiologic process. Augmentation of glycogenesis by the administered corticosteroids was obtained only in the presence of insulin, of either endogenous or exogenous origin. The absolute insulin-inadequate animal was incapable of this response. Only with this qualification applied can the glucosteroids be precisely considered as hepatoglycogenic. A prominent degree of glycogenesis was possible under circumstances in which insulin supply or its functional efficiency was reduced. Interpretation implies the satisfying of glycogenic needs in the midst of manifestations of "somatic" insulin inadequacy. Toward this accomplishment the insulin secretory tissue could seem anatomically well placed. E.A.W.

Fuller, Harvey L. (Baltimore, Md.): REDUCTION OF SERUM CHOLESTEROL IN HYPERCHOLESTEREMIC PATIENTS: EFFECT OF A POLYSORBATE 80-CHOLINE-INOSITOL COMPLEX. Maryland State Journal *8*:6-13, 1959.

Forty-one patients with hypercholesteremia associated with various pathologic entities were treated with a polysorbate 80-choline-inositol complex (Monichol). In all patients there was a significant fall in serum cholesterol; in general the higher the initial level, the greater the reduction achieved. S.S.

Gellis, Sydney S.; and Hsea, David Yi-Yung (Dept. of Pediatrics, Boston University Sch. of Med., Boston, Mass., and Northwestern University Medical School, Chicago, Ill.): THE INFANT OF THE DIABETIC MOTHER. A.M.A.J. Dis. Child. *97*:1-35, January 1959.

The authors summarize their own experiences with 934 diabetic pregnancies, as well as carefully reviewing the literature. They conclude that pregnancy failure is several times more frequent in the diabetic than in the normal woman, occurring principally after twenty-eight weeks of gestation, and is little altered except by experience and teamwork by any known therapeutic regimen, not excluding hormonal therapy. Only two maternal factors appear to affect fetal mortality: (a) the severity of maternal disease and (b) good diabetic control. Ideally, infants of diabetic mothers should be delivered between thirty-five and thirty-six weeks of gestation, thus eliminating both the risk of prematurity in early delivery and the risk of stillbirth in late delivery. The mode of delivery or type of

anesthesia does not seem to effect the outcome.

Clinically, respiratory distress is the principal complication. Pulmonary hyaline membrane and atelectasis are the only findings in two thirds of the infants who die. Hypoglycemia, infections and congenital anomalies are of minor importance in the neonatal mortality rate.

No specific therapeutic measure, such as antibiotics, mist, oxygen or cortisone is helpful in prevention or treatment of infants with hyaline membrane disease; and no rational treatment is likely until the pathogenesis of the disease is understood. R.L.J.

Gillespie, Luke; and Ferguson, James Henry (Harvard Med. Sch., Boston, Mass.; Dept. of Obst. & Gynec.; and Univ. of Miami Sch. of Med., Dept. of Obst. & Gynec., Miami, Fla.): DIABETES AND PREGNANCY. Am. J. Obst. & Gynec. 76:679-85, September 1958.

The case of a physically well, asymptomatic twenty-four-year-old primigravida, twenty-four weeks' pregnant, in whom glycosuria was found on three occasions, is presented to obtain the viewpoints of the two specialists as to establishing the diagnosis of diabetes mellitus, and as to the plan of management of the patient if the diagnosis of diabetes mellitus was established. Both consultants presented very similar views as to the patient's care: a glucose tolerance curve first, which, if abnormal, would establish the diagnosis of diabetes and exclude renal glycosuria. A carbohydrate-restricted diet might be tried first, and then insulin if diet alone was unsuccessful. A trial with Orinase was suggested but not advocated. Weight regulation including salt restriction, possibly with the use of diuretics such as acetazolamide or chlorthiazide; frequent checkups; delivery by pelvic route or cesarean section at thirty-seven to thirty-eight weeks, depending on obstetrical judgment, with care of the newborn as if the baby were premature, were agreed upon by both consultants. Differences were expressed as to the use of female sex hormone in the mother, and the use of glucose in the newborn.

The actual management of the case was much in accordance with the consultants' opinions. The diagnosis of diabetes was established by a glucose tolerance test. The patient was given insulin, and did well throughout pregnancy. At thirty-six weeks, a normal 3,720-gm. baby boy was delivered by cesarean section. The woman continued to receive insulin at the time of discharge. R.L.J.

Grollman, Arthur P.; and Gamble, James L., Jr. (Dept. of Pediat., Johns Hopkins University Sch. of Med., Baltimore, Md.): METABOLIC ALKALOSIS, A SPECIFIC EFFECT OF ADRENOCORTICAL HORMONES. Am. J. Physiol. 196:135-40, January 1959.

With appropriate balance experiments and skeletal muscle analysis, it was shown that: (1) Dogs placed on a potassium-restricted diet lost approximately 15 per cent of their muscle-cell potassium but did not develop alkalosis. (2) The administration of DCA, ACTH or cortisone to animals on a potassium-restricted diet evoked metabolic alkalosis which was sustained only as long as the hormones were administered. A prompt return to normal occurred despite continued potassium restriction. (3) Alkalosis induced by chloride withdrawal or by a cortisone administration during complete sodium restriction was not associated with evidence of potassium loss. These findings indicate that the increase in the serum bicarbonate induced by the steroids is a relatively direct effect, one that cannot be dependably related in a reciprocal fashion to the associated losses of body potassium. L.S.S.

Hartel, Gottfried (Wihuri-Forschungsinstitut und dem pharmakologischen Laboratorium der Arzneimittelfabrik "Laaketehdas Orion Oy," Helsinki, Finland): ÜBER DIE BEDEUTUNG DER SCHILDDRUSE FUR DAS AUFTRETEN VON ERHOHTEN SERUM CHOLESTEROLINWERTEN BEI RATTEN NACH FUTTERUNG VON N1-SULFANILYL-N2-N-BUTYLCARBAMID (BZ-55). Acta endocrinol. 29:267-72, October 1958.

The author had previously demonstrated an elevation of serum cholesterol in rats after oral administration of carbutamide. He repeated the experiments on groups of ten rats. One group received only the drug (in large doses of 0.5 gm. per kg. body weight). Other groups had thyroidectomy alone and combined with drug administration. The latter did not enhance the rise of serum cholesterol (and body weight) seen after thyroidectomy alone. He concluded that the drug effect was mediated through the thyroid by a thyrostatic effect. S.B.B.

John, Henry J. (Cleveland, Ohio): THE CORRELATION OF THE FALL OF BLOOD SUGAR WITH THE REDUCTION OF WEIGHT. J. Michigan M. Soc. 57:1415-18, October 1958.

Five cases of obesity are presented — patients who at the beginning showed a disturbance of carbohydrate metabolism of various degrees of severity. By placing them on a reducing diet, not only did they lose weight, but at the same time their carbohydrate metabolism improved — in fact, became normal and stayed thus. W.R.K.

Jones, O. Hunter (Charlotte Memorial Hosp., Dept. of Obst. & Gynec., Charlotte, N. C.): PREGNANCY WITH DIABETES MELLITUS COMPLICATED BY HYDRAMNIOS AND LOSS OF FETAL MOVEMENTS. Am. J. Obst. & Gynec. 76:817-20, October 1958.

The author describes two cases in diabetic women of pregnancy complicated by hydramnios, in which fetal movements decreased, or ceased, but fetal heart tones were normal. He briefly reviews the literature, and other authors' management of the problem; and concludes that the above is a grave sign, usually implying impending fetal death. When hydramnios is present he believes the pregnancy should be interrupted if the stage of pregnancy makes this feasible, before the above complication arises. R.L.J.

Joslin, Elliott P.; Lombard, Herbert L.; Burrows, Ruth E.; and Manning, Miriam D. (Joslin Clinic and New Eng. Deaconess Hosp., Boston, Mass.): DIABETES AND CANCER. New England J. Med. 260:486-88, March 5, 1959.

Based on data collected on 1,069 patients, it is concluded that diabetes does not predispose to cancer since the number of cases of cancer did not differ significantly from the expected value. S.S.

Jost, Franz (St. Vincent's Hosp., New York, N. Y.): BLOOD DYSCRASIAS ASSOCIATED WITH TOLBUTAMIDE THERAPY. J.A.M.A. 169:1468-69, March 28, 1959.

Severe, protracted, and probably lethal aplastic anemia occurred in a patient taking tolbutamide. This should in no way discourage the use of this effective and generally remarkably safe drug. It should, however, remind the physician of the possibility that mild and inconsequential reactions may occasionally assume grave proportions and alert him to the necessity of detecting them by frequent blood cell counts early in the course of treatment. B.F.K.

Kaiser, Irwin H.; and Goodlin, Robert C. (Dept. of Obst. & Gynec., Univ. of Minnesota Med. Sch., Minneapolis, Minn.): ALTERATIONS OF *p*H, GASES AND HEMOGLOBIN IN BLOOD AND ELECTROLYTES IN PLASMA OF FETUSES OF DIABETIC MOTHERS. Pediatrics *22*:1097-1109, December 1958.

The present study is based on observations of *p*H and content of gases in the blood, and of electrolytes in the plasma of eighteen diabetic mothers and their fetuses, the blood in the latter being obtained before the establishment of respiratory exchange at the time of delivery. The pregnant full-term diabetic, in absence of clinical metabolic disorder, differs little in above determinations from the normal. A diabetic in labor is apt to show evidence of only partially compensated metabolic acidosis. The fetus of the diabetic, however, frequently is found to have alterations in *p*H, pCO_2, oxygen saturation of blood, slight decrease in electrolyte concentration, slight increase in hemoglobin concentration. The changes in *p*H and pCO_2 are sufficient, in their effect on the dissociation of oxyhemoglobin, to account for the mean decrease in saturation without any change in pO_2. The biochemical alterations in the fetuses are out of proportion to maternal changes, are readily reversed when pulmonary ventilation is established, and are irregularly related to neonatal difficulty. These biochemical changes, per se, do not appear sufficient, except possibly in exaggerated form, to account for the known neonatal difficulties of the infants of diabetic mothers. The authors suggest that the cause may lie in the maternal placenta, and not in the mother or in the fetus. R.L.J.

Káldor, Antal; and Pogátsa, Gábor (3rd Dept. of Medicine, University of Budapest, Hungary): THE MODE OF ACTION OF ORAL ANTIDIABETIC COMPOUNDS. Lancet *1*:386, Feb. 21, 1959.

The authors report that tolbutamide inhibits the glycogenolytic effect of thyroxine, curare, and posterior-pituitary extract when given simultaneously to rats. These three substances appear to be similar only in their glycogenolytic action. Whether the glycogenolytic action is affected through the intervention of some other mechanism remains to be explored. In the control of diabetes, these substances possibly exert their effect by impeding glycogenolysis, thus "defending" the glycogen content of the liver. C.A.R.

Kempner, Walter; Peschel, Ruth Lohmann; and Schlayer, Clotilde (Duke Univ. Sch. of Med., Durham, N. C.): EFFECT OF RICE DIET ON DIABETES MELLITUS ASSOCIATED WITH VASCULAR DISEASE. Postgrad. Med. *24*:359-71, October 1958.

A report is given on 100 consecutive patients with diabetes mellitus associated with vascular disease who were treated with the rice diet. They were followed for from three months to eleven years, and the average period of observation was twenty-two months. The course of the disease can be favorably changed by intensive treatment with the rice diet. W.R.K.

Kershbaum, Alfred; Bassett, David R.; and Bellet, Samuel (Div. of Cardiology, Philadelphia Genl. Hosp., Philadelphia, Pa.): THE EFFECT OF ELECTRIC CONVULSIVE THERAPY ON SERUM LIPIDS. Am. J. M. Sc. *237*:341-44, March 1959.

The authors report upon the effect of electric convulsive therapy on serum cholesterol, phospholipids, and lipoproteins in fourteen psychiatric patients. No significant change in serum lipid levels was found in the majority of subjects, either as a result of anticipating electric convulsive therapy or of the actual convulsion. L.S.S.

Kirk, J. E.; Wang, I.; and Brundstrup, N. (Div. of Gerontology, Washington Univ. Sch. of Med., St. Louis, Mo.): THE GLUCOSE-6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITIES OF ARTERIAL TISSUE IN INDIVIDUALS OF VARIOUS AGES. J. Gerontology *14*:25-31, 1959.

A comparison of the mean activities of normal and arteriosclerotic tissue showed distinctly lower glucose-6-phosphate dehydrogenase values for the arteriosclerotic tissue, whereas the difference in 6-phosphogluconate dehydrogenase activity was only moderate. Studies were made on eighty-five samples of the aorta, seventy-eight samples of the pulmonary artery and forty-five samples of the coronary artery, obtained fresh at autopsy from individuals aged zero to eighty-five years. In the case of the aortic and coronary artery samples, the enzyme measurements were carried out both on normal and arteriosclerotic areas of the vessel wall. S.S.

Koszewski, Bohdan J. (Creighton Univ. Sch. of Med., Omaha, Neb.): THE TREATMENT OF DIABETIC COMA. Nebraska M. J. *43*:481-84, November 1958.

The author restates the principles and technic of management of diabetic coma. While he dwells largely upon treatment of the emergency, he reiterates the known facts about cause and prevention of this complication which carries such a grave prognosis and which demands the most prompt and skillful management. W.R.K.

Krall, Leo P.; and Bradley, Robert F. (Joslin Clinic and New England Deaconess Hosp., Boston, Mass.): CLINICAL EVALUATION OF FORMAMIDINYLIMINOUREA, A NEW BIGUANIDE ORAL BLOOD SUGAR LOWERING COMPOUND: COMPARISON WITH OTHER HYPOGLYCEMIC AGENTS. Ann. Int. Med. 50:586-613, March 1959.

This is a study of the treatment of human diabetes with formamidinyliminourea (DBI). The group consisted of 173 patients varying widely in age, duration of diabetes and insulin requirements. The drug was used to replace exogenous insulin in patients already treated by insulin or to substitute for insulin in new, untreated diabetics. A blood sugar lowering effect was produced in 88 per cent. Successful therapy was accomplished in 62 per cent and the drug could be used for long-term (average one year) therapy in 47 per cent.

Therapeutic success was related mainly to the (shorter) duration of the disease. The average maintenance dose was 150 mg. per day. The greatest impediments to success were the gastrointestinal side effects. These were quickly reversed by omission or decrease in quantity of the drug and were not considered toxic manifestations. No actual toxicity was encountered.

The authors gave as the main indication for the drug its use with insulin for stabilization of labile juvenile diabetes and the total replacement of insulin in the milder, maturity-onset diabetes. It was effective (62 per cent) in a group of thirty-one diabetics previously treated unsuccessfully with sulfonylureas.

A review of the background of oral therapy of diabetes as well as the mechanism of action of DBI is included. S.B.B.

Kushner, Daniel S.; and Szanto, Paul S. (Cook County Hosp., Chicago, Ill.): DIABETES MELLITUS COMPLICATED BY ENTEROCOCCIC MENINGITIS. J.A.M.A. *169*:949-55, Feb. 28, 1959.

From the findings at necropsy diabetes was explained by a combination of chronic, relapsing pancreatitis involving the head of the pancreas with a silent cystadenoma which caused an extensive atrophy of the distal portion of the pancreas. The

patient developed acute exacerbation of chronic pancreatitis which led to peripancreatic abscess precipitating diabetic coma. This abscess, in turn, led to pyelophlebitic abscesses, either of which may have resulted in a suppurative meningitis. Whenever a patient in diabetic coma develops symptoms of meningitis, this possibility deserves consideration. B.F.K.

Landau, Bernard R.; and Lubs, Herbert A. (Dept. of Health, Ed., and Welfare, P.H.S., Nat'l. Cancer Inst., Endocrinology Branch, Bethesda, Md.): ANIMAL RESPONSES TO 2-DEOXY-D-GLUCOSE ADMINISTRATION. Proc. Soc. Exper. Biol. & Med. 99:124-27, October 1958.

The response of mice, rats, and rabbits to acute and chronic administration of 2-deoxy-D-glucose (2-DG) is presented. Mice and rabbits were given large doses of 2-deoxy-D-glucose intravenously and had symptoms similar to those during insulin hypoglycemia. It is suggested that the symptoms were the result of a cellular glucopenia secondary to inhibition by 2-DG of glucose utilization. Rats receiving 2-DG chronically by subcutaneous administration had elevated blood glucose concentrations and weight loss. On discontinuation of 2-DG administration blood glucose concentrations returned to control values. The intravenous administration of small doses of 2-DG to rabbits resulted in a rapid rise in blood glucose concentrations which remained elevated for at least five hours. Glucose tolerance tests done shortly after 2-DG administration resulted in a much slower return of the elevated glucose concentrations to normal as compared to the control tolerance tests. G.J.H.

Larson, Carroll B. (State University of Iowa, Coll. of Med., Iowa City, Ia.): FOOT COMPLICATIONS IN DIABETES. J. Iowa M. Soc. 48:187-91, April 1958.

The feet of patients with diabetes, however mild, should be examined repeatedly for early signs of pressure points, ulcers or neuropathy. Every patient should be instructed to:

1. Report first signs of pressure redness or open sores.
2. Abstain from walking on a sore foot.
3. Use no heaters, drugs or chemicals without a doctor's order.
4. Control the diabetes carefully by means of diet and insulin as prescribed.

When ulcers have developed, the treatment should be immediate bed rest and alternating elevation, combined with local cleanliness to prevent infection. Antibiotics are helpful, but not a substitute for nonweight-bearing and cleanliness. No special therapy to the ulcer is necessary. Once the ulcer has healed, recurrence must be prevented by correcting foot mechanics, and eliminating pressure points such as cock-up toes through the use of pads, proper shoes and minor operative procedures if necessary. In the case of uncontrolled infections, surgical excisions should be planned in an attempt to gain primary closure. Surgery should be preceded by a careful vascular survey, since obvious vascular insufficiency would demand amputation at the proper level. W.R.K.

Lawrence, A. M.; Contopoulos, A. N.; and Simpson, M. E. (Dept. of Anat. and Inst. of Experimental Biology, Univ. of Calif., Berkeley, Calif.): PITUITARY AND PLASMA BIO-ASSAY FOR TROPHIC HORMONES IN THE ALLOXAN-DIABETIC RAT. Proc. Soc. Exper. Biol. & Med. 99:35-38, October 1958.

The gonadotrophic, thyrotrophic, and growth-hormone content of anterior pituitaries from female rats rendered diabetic by alloxan was determined by bio-assay in hypophysectomized female rats. Trophic hormones in blood plasma were also determined. The only difference found between the alloxan diabetic rats and the control rats was a reduction in growth hormone content of the pituitary. G.J.H.

Lazarus, Sydney S.; and Volk, Bruno W. (Isaac Albert Res. Inst. of Jewish Chronic Disease Hosp., Brooklyn, N. Y., and Dept. of Pathology, Albert Einstein Coll. of Med., Bronx, N. Y.): GLYCOGEN INFILTRATION ("HYDROPIC DEGENERATION") IN THE PANCREAS: A REVIEW. A.M.A. Arch. Path. 66:59-71, July 1958.

The problem of "hydropic degeneration" in the pancreas is reviewed. With present knowledge it should be recognized that this term is a misnomer and that this lesion should rather be called glycogen infiltration. It has been found that in the normal dog as well as in human and experimental diabetes glycogen infiltration may frequently be present only in ductular epithelium. On the other hand, β-cell glycogen infiltration occurs as a comparatively late phenomenon which depends on the duration and severity of the diabetic state. Taken in conjunction these observations are at variance with the heretofore generally accepted view that "hydropic degeneration" is an expression of functional exhaustion of β cells. In the light of this, the linking of β-cell degranulation and vacuolation as sequential events in the evolution of β cell degeneration in diabetes can no longer be justified. These two lesions should rather be considered as independent manifestations of hyperglycemia, the β cell degranulation indicating increased insulinogenesis and the glycogen infiltration being merely one component of the generally increased glycogen deposition in diabetes. Furthermore, since glycogen at other sites does not cause cell destruction, it is questioned whether infiltration of this material in β cells is responsible for the development of the various forms of metadiabetes. E.A.W.

Leith, W.; and Beck, J. C. (McGill Univ. Clin., Royal Victoria Hosp., Montreal, P. Q., Canada): THE USE OF PHENMETRAZINE HYDROCHLORIDE (PRELUDIN) IN THE OBESE DIABETIC. Canad. M. A. J. 79:897-98, Dec. 1, 1958.

A double blind test of the efficacy of phenmetrazine hydrochloride was conducted among thirty adult diabetics. Among these were patients treated with diet alone and patients treated with insulin as well. Of twenty patients who cooperated, fifteen showed weight loss due to the drug. Among the fifteen patients with weight reduction, no effect on the blood sugar level or its fluctuation, or on insulin requirements was observed. S.B.B.

Linton, Robert R. (Harvard Med. Sch., Boston, Mass.): PERIPHERAL VASCULAR DISEASES. New England J. Med. 260:272-77, 322-27 and 370-75, Feb. 5, 12 and 19, 1959.

A comprehensive current review. S.S.

Lonergan, Peggy; and Robbins, Stanley L. (Boston Univ. Sch. of Med. and the Mallory Institute of Pathology, Boston, Mass.): ABSENCE OF INTERCAPILLARY GLOMERULOSCLEROSIS IN THE DIABETIC PATIENT WITH HEMOCHROMATOSIS. New England J. Med. 260:367-69, Feb. 19, 1959.

The clinical records and postmortem microscopic renal sections of sixty-two patients with so-called diabetes incident to hemochromatosis were reviewed. In twenty-one of these patients, the onset of diabetes was five to nineteen years before death. No lesions of intercapillary glomerulosclerosis were found in these cases, and such an absence represents a statistically valid

difference from a comparable group of cases of diabetes mellitus. It is proposed that the diabetes associated with hemochromatosis differs from diabetes mellitus in not predisposing to renovascular disease and supports the thesis that the derangements leading to vascular lesions in the patient with diabetes mellitus are not necessarily directly related to the defect in carbohydrate metabolism. S.S.

MacGregor, Kenneth H. (Dept. of Surg., Brooklyn Hosp., Brooklyn, N. Y.): GASTROJEJUNOCOLIC FISTULA WITH ASSOCIATED ISLET CELL TUMORS OF THE PANCREAS. Am. J. Surg. 96:98-101, July 1958.

A case is reported in which peptic ulceration occurred in a female having a islet cell tumor of the body and tail of the pancreas. She had had a gastroenterostomy for marginal ulcer several years before discovery of the tumor. A.R.C.

McPherson, Harry T.; Werk, Emile E., Jr.; Myers, Jack D.; and Engel, Frank L. (Dept. of Med., Duke Univ. Sch. of Med., Durham, N. C.): STUDIES ON KETONE METABOLISM IN MAN. II. THE EFFECT OF GLUCOSE, INSULIN, CORTISONE AND HYPOGLYCEMIA ON SPLANCHNIC KETONE PRODUCTION. J. Clin. Invest. 37:1379-93, October 1958.

In a series of twenty-seven "mildly ill" hospital patients the net splanchnic ketone production was studied in the fasting state with the use of hepatic venous catheterization after intravenous infusion of sodium octoanate. The addition of glucose alone significantly reduced ketone production; the further addition of insulin did not enhance this effect. The mechanism was believed to be suppression of endogenous fatty acid mobilization or catabolism.

Insulin hypoglycemia did not effect utilization of infused octoanate in spite of elevation of peripheral venous blood ketone levels. Cortisone pretreatment did not modify the effect of insulin hypoglycemia. S.B.B.

Makman, Maynard H.; Makman, Richard S.; and Sutherland, Earl W. (Dept. of Pharmacol., Sch. of Med., Western Reserve University, Cleveland, Ohio): PRESENCE OF A GLUCAGON-LIKE MATERIAL IN BLOOD OF MAN AND DOG. J. Biol. Chem. 233:894-99, October 1958.

Blood fractions were assayed for glucagon activity in a dog liver homogenate system by means of their ability to convert dephospho-liver phosphorylase to liver phosphorylase. Plasma glucagon activity in dog or man was estimated to be about 7 μg. per 100 ml. of plasma in terms of its crystalline glucagon equivalent. This represents a total circulating amount of approximately 0.2 mg. in man. The material stimulated glucose output from liver slices and was inactivated by trypsin and "insulinase" extract. Following injection of glucagon into the portal vein, it appeared in the femoral vein blood. This indicates that it is secreted by the pancreas. No difference between dog pancreatic and femoral venous blood content was noted. A.R.C., JR.

Malinow, Manuel R. (Dept. of Physiol., Sch. of Med., University of Buenos Aires, Argentina): THE EFFECT OF INSULIN ON THE ELECTRICAL ACTIVITY OF ISOLATED VENTRICULAR MUSCLE OF THE RAT. Acta physiol. latinoam. 8:125-28, 1958.

The effect of insulin on the isolated ventricular muscle of the rat has been studied. No changes in the resting potential nor in the overshoot of the action potential were seen but the duration of the repolarization process was shortened. W.R.K.

Marble, Alexander (Harvard Med. Sch., Boston, Mass.): PRESENT STATUS OF THE ORAL HYPOGLYCEMIC AGENTS. Nebraska M. J. 43:491-95, November 1958.

In a group of 594 carefully selected diabetics, 70 per cent were satisfactorily controlled. The author emphasizes that this does not mean that 70 per cent of all diabetics can use the oral hypoglycemic agents but rather that of those patients who meet rigid criteria for the use of sulfonylurea compounds 70 per cent may have a satisfactory response. W.R.K.

Monaco, Robert N.; Anderson, George E.; and Kaulbach, Maximilian (Dept. of Med. of the Brooklyn Hosp., Brooklyn, N. Y.): INSULIN SENSITIVITY IN ORGANIC HYPERINSULINISM: DIAGNOSIS OF INSULINOMA. Metabolism *VIII*:49-59, January 1959.

The authors demonstrate a marked lack of insulin sensitivity in organic hyperinsulinism following removal of insulinomas. They compare this phenomenon to the impaired sensitivity to insulin in diabetic patients treated with chronic overdosage of insulin. Using glucagon-free insulin and employing the six-minute insulin activity test as described by Anderson and Fribourg, the data are both clear-cut and diagnostic. Complete reversion to the insulin sensitive state occurred in both the chronic over-treated diabetic and the patients with organic hyperinsulinism after withdrawal of excessive doses of insulin in the former and removal of the islet cell tumors in the latter.

The authors suggest that the six-minute test may aid in the differential diagnosis of organic hyperinsulinism from other types of hypoglycemia. The advantages of the six-minute test as contrasted to the other types of insulin tolerance tests are discussed. R.W.S.

Mulder, Donald W.; and Rushton, Joseph G. (Sect. of Neurol., Mayo Clinic and Foundation, Rochester, Minn.): HYPERINSULINISM: RARE CAUSE OF EPILEPSY. Neurology 9:288-89, April 1959.

Hyperinsulinism and epilepsy are both clinically characterized by episodic, cerebral disturbances. In this group of ninety-one patients with hyperinsulinism, seizures were rarely the initial symptom and were never the only, or even the principal, symptom. The diagnosis can be suspected in most instances on the basis of the patient's description of spells and can be confirmed by (1) precipitating an attack by fasting up to seventy-two hours; (2) obtaining low levels of blood sugar during an attack; and (3) relieving symptoms by administration of glucose. W.R.K.

Narahara, H. T.; and Williams, Robert H. (Dept. of Med., University of Washington Sch. of Medicine, Seattle, Wash.): EFFECT OF PROTEIN ADDED IN VITRO UPON INSULIN DEGRADATION AND GLUCOSE UPTAKE BY MUSCLE. J. Biol. Chem. 233:1034-40, November 1958.

Muscle of rat diaphragm was shown to be capable of destroying insulin labeled with I^{131} during incubation in vitro. Certain proteins may inhibit insulin degradation by acting as substrates for a proteolytic enzyme system. Insulin degradation was also inhibited by corticotropin, oxidized insulin, α-casein, glucagon and growth hormone. Glucose uptake by rat diaphragm was augmented by these substances. These hormones and protein derivatives may enhance glucose utilization by sparing insulin from destruction. A.R.C., JR.

Nomland, Ruben (State University of Iowa Coll. of Med., Iowa City, Ia.): DISEASES OF THE SKIN IN DIABETES. J. Iowa M. Soc. *48*:191-93, April 1958.

Diabetics may have the same skin diseases as other people, but there are several groups of diseases which are limited to, or are more serious in, the diabetic. Greater susceptibility to and more serious cases of both monilial and pyogenic infections are found in the diabetic. Disturbances of fat metabolism occur, with accompanying skin lesions. Xanthoma diabeticorum is associated with hyperlipemia and eruption of xanthomatous nodules, whereas necrobiosis lipoidica diabeticorum is unassociated with systemic lipoid changes and presents a characteristic eruption on the shins, with microscopic findings typical of necrobiosis and fat imbibition. W.R.K.

Paley, Ronald G.; and Scott, Mary H. (Dept. of Med., Univ. of Leeds, England): SEVERE INSULIN LIPODYSTROPHY AS A POSSIBLE CAUSE OF INSTABILITY IN DIABETICS. Brit. M. J. 2:1331-34, Nov. 29, 1959.

The authors studied six unstable diabetic patients with severe atrophic lipodystrophy and three patients with severe hypertrophic lipodystrophy, to determine if insulin lipodystrophy was a factor in their instability. No pattern of significant differences was observed in the blood sugar curves obtained from different injection sites, and therefore the authors feel that lipodystrophy cannot be regarded as a cause of diabetic instability. Regarding the belief that intramuscular injections of insulin may cause tissue necrosis, it is of interest that no untoward effects occurred from such injections given for four to seven days. C.A.R.

Pedowitz, Paul; and Shlevin, Edmund L. (Dept. Obst., Gynec., Med. at Jewish Hosp., Brooklyn; State Med. Center and the Kings Co. Hosp., New York, N. Y.): DIABETES MELLITUS AND PREGNANCY: 1932-57. AN ANALYSIS OF THE FACTORS RESPONSIBLE FOR THE IMPROVED FETAL SALVAGE DURING THE CURRENT DECADE. Am. J. Obst. & Gynec. 76:417-24, August 1958.

Management of 141 viable pregnancies in diabetic patients from 1932 to 1949 is compared to the present series of 219 cases extending from 1950 through 1957. The improved fetal salvage (8.7 per cent) in the present series as compared to 22 per cent in the previous series is attributed to better management and patient cooperation. In the present series uncomplicated cases were delivered about three weeks prior to the estimated confinement date, since neonatal death at this period is almost identical with that at term. Earlier delivery than this sharply increases the neonatal death rate as prematurity increases. Elective cesarean section is felt to be the best method of delivery when the cervix is unfavorable, and was utilized in 65 per cent of the present series of cases. Of these, fifty-seven of the 219 had previous sections as an indication. The authors feel that pregnant patients with abnormal glucose tolerance curves should be treated as diabetics since the high fetal wastage and embryopathology are similar to those with diabetes. Fasting blood sugars and urine specimens are not deemed adequate for diagnosis. Insulin is usually necessary later in pregnancy though diet may be adequate in the first trimester. R.L.J.

Randle, P. J.; and Taylor, K. W. (Dept. of Biochemistry, Univ. of Cambridge and Addenbrookes Hosp., Cambridge, England): INSULIN IN BLOOD. Endokrinologie *36*:211-16, July 1958.

The uptake of glucose by the isolated diaphragm has proved a simple and sensitive method for assaying insulin. It appears to be also a suitable method for the assay of serum or plasma insulin. The use of the in vivo method which is based on the blood sugar response of suitably sensitive animal preparations to injected serum or plasma is seriously limited by the difficulty of preparing and maintaining the test animals. The in vitro (diaphragm) method still has, however, certain shortcomings which must be eliminated before reliable assays in plasma and serum can be done. The principle disadvantage is its lack of specificity since plasma appears to contain under certain circumstances factors other than insulin, which can stimulate the uptake of glucose by isolated diaphragm (such as crystalline bovine serum albumin, salicylate, etc.). Moreover, blood may contain factors which antagonize or bind insulin to antibodies and thus render it biologically inactive. Ancillary methods will have to be developed not only to extract all the insulin present in the plasma or serum, but also to provide information about the form and biological activity of this blood insulin. Plasma protein fractionation methods have already proved to be most useful in this respect. Zone electrophoresis, ion-exchange resins and treatment with acid alcohol also may help to separate and extract insulin in biologically active form from plasma which appeared to be void of insulin activity. M.G.G.

Reis, Ralph A.; DeCosta, Edwin J.; and Gerbie, Albert B. (Dept. of Obst. and Gynec., Passavant Memorial Hosp., and Northwestern Med. Sch., Chicago, Ill.): PREGNANCY IN THE DIABETIC WOMAN. Am. J. of Obst. & Gynec. 76:1148-50, November 1958.

The authors reevaluate their previous recommendations in the management of diabetic pregnant women. Careful evaluation of the diabetic woman prior to or early in pregnancy is basic, with attention to the cardiovascular-renal system. Use of long-acting insulin, notably NPH, on a twelve-hour basis with supplements of regular insulin at six-hour interims shortens predelivery hospitalization. They still prefer to maintain a controlled glycosuria, do not advocate the use of female sex hormones, commenting on the masculinization of the female fetus with this therapy. They adhere less rigidly to prophylactic oral glucose of the newborn since reports of fetal salvage without this measure are comparable to theirs; however, care of the newborn as a premature infant is most important. R.L.J.

Riecker, G.; and Bubnoff, M. von. (1st Dept. of Med., Univ. of Munich, Germany): ON THE INTRACELLULAR WATER AND ELECTROLYTE METABOLISM. STUDIES ON ERYTHROCYTES. THIRD REPORT: WATER AND ELECTROLYTE CHANGES IN THE INTRACELLULAR SPACE AFTER GLUCOSE INFUSIONS. Klin. Wchnschr. 37:18-23, Jan. 1, 1959.

The distribution of water, Na, K, and glucose between intra- and extracellular fluid space was determined in twenty-two healthy individuals after intravenous infusions of 5 to 25 per cent glucose solutions. The intracellular changes in the erythrocytes were estimated by means of the chloride-distribution method of Elkinton and Danowski. The increase of the extracellular glucose concentration and the subsequent increased osmolarity of the serum causes an inflow of water into the intracellular space of the erythrocytes and an outflow of K and Na into the extracellular space. Estimation of the glucose concentration shows a glucose transport into the cells. Thus the intracellular osmolarity increases and explains the influx of water into the cell. This process is, however, counteracted in part by the electrolyte migration in the opposite direction.

Statistical evaluation of the data shows that under the described experimental conditions the water and electrolyte changes in the erythrocytes are identical with those in the other intracellular compartments of the body (German). M.G.G.

Root, Howard F.; Mirsky, Stanley; and Ditzel, Jörn (Joslin Clin. and The New England Deaconess Hosp., Boston, Mass.): PROLIFERATIVE RETINOPATHY IN DIABETES MELLITUS: REVIEW OF EIGHT HUNDRED FORTY-SEVEN CASES. J.A.M.A. *169*:903-09, Feb. 28, 1959.

In an analysis of 847 consecutive cases of proliferative retinopathy among diabetic patients at the Joslin Clinic, no patient was found with this condition who had had good control of diabetes with adequate diet and insulin therapy from the onset of the diabetes. The age at the onset of diabetes proved of greatest significance, in that diabetes developing early in life is severe, whereas diabetes developing late in life is, in general, mild.

Early diagnosis and continuous control of diabetes may be of maximum importance in postponing and preventing proliferative retinitis, since many patients with onset in childhood are now known to have survived diabetes for periods of twenty to thirty years without any evidence of retinal hemorrhages when diabetic treatment has been adequate. The average duration of diabetes in this group at the time retinitis proliferans was recognized was 17.6 yr., although it was impossible to correct the errors which are due to late or delayed diagnosis. The influence of sex and heredity appeared no different in diabetic patients with proliferative retinopathy than in those without.

The association of proliferative retinopathy and malignant diabetic nephropathy, or glomerulosclerosis of diabetes, is more intimate. Hypertension and albuminuria, although frequently absent at the onset, almost invariably appeared within months or years after the retinopathy. Causes of death were predominately due to the diabetic nephropathy. Uremia was present in more than 50 per cent of the patients. In the older group, although chronic glomerulosclerosis had been present, the terminal event was in many instances coronary occlusion.

The association of retinitis proliferans (proliferative retinopathy) with diabetic nephropathy and premature atherosclerosis in the young patient with severe diabetes further identifies this condition as one of the specific products of insulin deficiency and of the other metabolic defects which constitute the disease diabetes mellitus. The postponement or prevention of proliferative retinopathy is possible by intensified efforts to find diabetes early and to provide for its continuous adequate treatment by skillful use of insulin and of dietary measures. R.F.K.

Rubin, Alan (Dept. of Obst. and Gynec., Univ. of Pennsylvania, Sch. of Med., Philadelphia, Pa.): STUDIES IN HUMAN REPRODUCTION: II. THE INFLUENCE OF DIABETES MELLITUS IN MEN UPON REPRODUCTION. Am. J. Obst. & Gynec. *76*: 25-29, July 1958.

One hundred and ninety-eight men attending outpatient diabetes clinics were interviewed with respect to their reproductive histories, using as controls men attending a dental clinic and excluding those in both groups who had wives with diabetes. Twenty-five per cent of subjects age thirty to thirty-four were impotent and 54 per cent of those fifty to fifty-four years of age. Impotence was not related to age at onset of diabetes, duration, severity, vascular or neurologic complications. The diabetic men reported 16 per cent abortions in their wives as compared to 10 per cent for controls. Diabetics did not differ from controls in incidence of conceptions, premature births, stillbirths, malformations, sex ratio of offspring and birth weights. R.L.J.

Rubin, Alan; and Murphy, Douglas P. (Dept. Obst. & Gynec.; Gynecean Hosp. Inst. for Gynec. Res. of Sch. of Med. and Hosp. of Univ. of Philadelphia, Pa.): STUDIES IN HUMAN REPRODUCTION: III. THE FREQUENCY OF CONGENITAL MALFORMATIONS IN THE OFFSPRING OF NONDIABETIC AND DIABETIC INDIVIDUALS. J. Pediat. 53:579-85, November 1958.

The authors cite the problems involved in accurately estimating the malformation rate in infants of diabetic mothers, most important being: (a) the lack of a comparable control series in nondiabetic women; (b) the higher death rate (at least three times greater) of babies of diabetic women, a fact which is, according to Whites' series, not due to congenital anomalies; (c) therefore the greater care in observation in babies of diabetic mothers, and in all probability, a greater autopsy rate. In addition, there is lack of uniformity in defining a congenital anomaly (e.g., some include hydroceles, and others do not).

The incidence of anomalies in babies of diabetic mothers ranges in the literature from 0 to 16 per cent with an average of 5.3 per cent; in the infants of the general population, 2 to 3 per cent. A series of 140 diabetic men with 463 offspring exhibited (by interview of fathers, not examination of infants) no greater incidence of congenital malformation than in 350 offspring of 154 nondiabetic men. R.L.J.

Scheiffarth, F.; Frenger, W.; and Mockel, G. (From the Dept. of Med., University Erlangen, Germany): SEROLOGICAL STUDIES ON THE NATURE OF THE INSULIN ANTIBODIES. Deutsche Med. Wchnschr. *84*:177-80, Jan. 30, 1959.

Passive or indirect hemagglutination reactions with washed sheep erythrocytes, pretreated with tannic acid, were carried out with the serum of 110 diabetics and 133 controls. Regular insulin and crystalline insulin were employed as antigens; in addition, extracts of human livers were used as controls. All of the diabetics were under insulin treatment with daily dosages varying between 20 and 200 U.; two of the patients were insulin-resistant, twenty-seven had severe complications, such as cirrhosis of the liver, hemochromatosis, cholangitis, nephritis, exudative pleuritis, gangrene, or pyodermia and abscesses. Sixty-two of the control patients suffered from similar processes which are characterized frequently by dysproteinemia and hyperglobulinemia. A positive indirect hemagglutination reaction to both insulin and liver extract was found in the sera of the twenty-seven diabetics with complicating diseases as well as in the sera of the sixty-two nondiabetic control cases suffering from similar diseases. The reactions were negative in the sera of eighty-three uncomplicated diabetics and the seventy-one healthy controls. The two insulin-resistant patients showed positive reactions but had complicating diseases causing hyperglobulinemia. No relationship was found between insulin requirement and the serological reaction. The authors conclude from their observations that the demonstrated agglutinating serum factor does not possess the properties of a specific insulin antibody and that its development depends rather on the serum protein abnormalities (dysproteinemia) which were present in the sera of both the diabetic and nondiabetic patients with positive reactions. M.G.G.

Serif, G. S.; Stewart, C. J.; Nakada, H. I.; and Wick, A. N. (Scripps Clinic and Res. Foundation, La Jolla, Calif.): INFLUENCE OF 6-DEOXY-6-FLUOROGLUCOSE ON GLUCOSE UTILIZATION IN RAT EPIDIDYMAL ADIPOSE TISSUE AND RAT DIAPHRAGM. Proc. Soc. Exper. Biol. & Med. 99:720-22, December 1958.

It has been demonstrated that 2-deoxy-glucose inhibits glucose metabolism and exerts its effect at a number of sites in the metabolic cycle. The action of 6-deoxy-6-fluoroglucose on the oxidation of glucose was compared to that of deoxy-glucose in rat epididymal adipose and rat diaphragm tissue. It was found that 6-deoxy-6-fluoroglucose inhibited glucose metabolism at all concentrations but approached a maximum inhibition of 50 to 75 per cent at the highest concentrations. The inhibition obtained with 2-deoxy-glucose appeared to approach 100 per cent inhibition of glucose oxidation in these tissues. These results have been interpreted to mean that two or more pathways previous to the hexokinase reaction appear to exist for oxidation of glucose in these tissues, and that only one of these pathways is inhibited by the 6-deoxy-fluoroglucose. G.J.H.

Shepherd, Herndon G., Jr.; and McDonald, Hugh J. (Loyola University, Chicago, Ill.): BINDING AFFINITY OF PURIFIED PLASMA PROTEINS FOR PHENETHYLBIGUANIDE, AN ORAL HYPOGLYCEMIC COMPOUND. Clin. Chem. 4:496-509, December 1958.

A colorimetric method has been adapted to the quantitative determination of the oral hypoglycemic compound, phenethylbiguanide. This method was useful in studying the binding affinity of purified bovine plasma albumin and gamma globulin for the hypoglycemic agent. Equilibrium dialysis studies, which measure the decrease in thermodynamic activity of the bound cation, reveal that there is no significant interaction between phenethylbiguanide and the two native proteins. Consequently, the two plasma proteins are assumed to exert no buffering action in controlling the plasma concentration of the drug. The adherence of phenethylbiguanide to Beer's Law indicates that the compound exists in the monomeric state in aqueous solution below 10^{-4} molar. W.R.K.

Silver, Henry K. (Dept. of Pediat., Univ. of Colorado Sch. of Med., and Yale Univ. Sch. of Med.): BUTANOL-EXTRACTABLE IODINE VALUES IN THE OFFSPRING OF DIABETIC WOMEN. A.M.A. J. Dis. Child. 96:144-47, August 1958.

The butanol extractable iodine content in five of seven babies of diabetic mothers was abnormally low, as compared to the levels in twenty-four infants of normal mothers. The author speculates that it may be due to excess production of thryoid hormone by the mother and mentions other authors' theories of increased fetal adrenocortical activity with thyroid inhibition, or secondary liver changes. R.L.J.

Simpson, John R. (Harper Hosp., Detroit, Mich.): STEROID DIABETES—CASE REPORT. Harper Hosp. Bull. 15:43-51, March-April 1957.

"Steroid diabetes" is characterized by the triad of: (1) insensitivity to insulin; (2) decreased glycosuria upon fasting in the absence of insulin; and (3) development of a negative nitrogen balance. An illustrative case is reported which highlights these characteristics and also presents the additional complication of hypokalemic, hypochloremic alkalosis. The interesting cardiac arrhythmias that appeared secondary to the hypokalemia are also discussed. Discontinuance of large doses of ACTH and correction of the hypokalemia resulted in prompt amelioration of the "steroid diabetes" and the metabolic alkalosis. W.R.K.

Skom, Joseph H.; and Talmage, David W. (Dept. of Med., Univ. of Chicago, Chicago, Ill.): NONPRECIPITATING INSULIN ANTIBODIES. J. Clin. Invest. 37:783-86, June 1958.

A method is described by which insulin bound by serum could be measured quantitatively by a new immunological method. This depends on the fact that insulin bound to its gamma globulin antibody fraction of human serum does not dissociate when the latter is precipitated. Precipitation is accomplished in vitro by the use of rabbit antihuman gamma globulin. S.B.B.

Skom, Joseph H.; and Talmage, David W. (Dept. of Med., Univ. of Chicago, Chicago, Ill.): THE ROLE OF NONPRECIPITATING INSULIN ANTIBODIES IN DIABETES. J. Clin. Invest. 37:787-93, June 1958.

A new immunological method was used to measure the serum-bound insulin of forty-eight patients. Of these there were twenty-five diabetics under current insulin therapy and ten whose insulin had been discontinued. Binding was not demonstrable in the serum of patients without previous insulin treatment. It appeared as soon as five weeks after onset of therapy and persisted up to three years after cessation. The titer was related to duration of treatment. In two patients with a change in insulin requirement no change in titer was demonstrable. S.B.B.

Sobel, Gerald W.; and Levine, Rachmiel (Dept. of Metabolic and Endocrine Res., Michael Reese Hosp., Chicago, Ill.): SULFONYLUREAS IN DIABETES MELLITUS. J. Am. Dietet. A. 34:1072-74, October 1958.

This article is a brief summary of the development of the sulfonylureas, their postulated role in increasing the production of endogenous insulin, and their place in the treatment of the mild adult diabetic, especially the elderly, those with visual difficulties and those in whom insulin reactions are to be avoided. R.L.J.

Somogyi, Michael (Jewish Hosp., St. Louis, Mo.): DIABETOGENIC EFFECT OF HYPERINSULINISM. Am. J. Med. 26:192-98, February 1959.

The author presents an interpretation concerning the paradoxical deterioration of carbohydrate tolerance in persons with organic hyperinsulinism and offers the hypothesis that excess insulin action induces a stimulation of the adrenal-pituitary system which eventually outstrips insulin action. The transitory diabetic, after surgical removal of the islet cell adenoma, is a manifestation of adrenalin diabetes. Artificial hyperinsulinism, produced by administering insulin to nondiabetic persons or its excessive use in diabetic patients, is accompanied by the same abnormalities since hypoglycemic episodes activate the diabetogenic factors. Evidence is further presented showing the greater the excess of insulin and the longer the subject is exposed to it, the greater is the diabetogenic effect. R.W.S.

Somogyi, Michael (Jewish Hosp. of St. Louis, St. Louis, Mo.): EXACERBATION OF DIABETES BY EXCESS INSULIN ACTION. Am. J. Med. 26:169-91, February 1959.

In this very informative timely paper, the author stresses the fact that induced hypoglycemia begets hyperglycemia. Excessive glycosuria is an aftermath of hypoglycemia. Brittle and unmanageable diabetics who are given increasing doses of insulin to overcome this glycosuria develop more and more hypoglycemic episodes with ensuing tidal waves of glycosuria. Even asymptomatic hypoglycemic episodes undoubtedly commonly

produce some effects. The complete avoidance of hypoglycemia by an increasing carbohydrate intake with intermediate feedings and with gradual reduction in insulin dosage uniformly results in stabilization and rehabilitation oftentimes with little or no insulin being required. The author presents unchallengeable proof of his claims and compares the insulin intoxication to an alarm reaction with stimulation of the pituitary-adrenal system with a resulting increased production of insulin-antagonistic hormones from the adrenal cortex and medulla. R.W.S.

Somogyi, Michael; and Goldwasser, H. V. (Jewish Hosp. of St. Louis, St. Louis, Mo.): QUANTITATIVE RELATIONSHIP BETWEEN INSULIN DOSAGE AND AMOUNT OF CARBOHYDRATES UTILIZED IN DIABETIC PERSONS. Am. J. Med. *26*:165-68, February 1959.

The authors discuss the quantitative relationship between insulin dosage and the amount of carbohydrates utilized by diabetic patients. The article summarizes the attempts made by various authors, demonstrates the different figures based on their own experience, and the consequent confusion resulting from the widely divergent opinions. The reason for these differences is clarified. They conclude that the magnitude of the G/I ratio is largely dependent on the quantitative relationship between the amount of carbohydrate ingested and the amount of insulin injected, a relationship expressed as R which represents the glucose available in diet/insulin dosage. The G/I ratio increases when R increases and shrinks when R decreases. Thus the range of variability under the influence of a changing R ratio is very great. Insulin action evidently yields diminished returns as R decreases and helps to explain the great divergencies in the glucose equivalent of insulin. R.W.S.

Sotto, Luciano S.; Heimback, Dennis P.; and Siegner, Allan W. (Dept. of Obst. and Gynec., Millard Fillmore Hosp., Buffalo, N. Y.): UNSUSPECTED "PREDIABETIC STATE" AND PREGNANCY. Am. J. Obst. & Gynec. 76:425-31, August 1958.

The authors studied glucose tolerance curves in 300 patients within twenty-four hours of delivery, of which 198 had factors suggestive of the prediabetic state: babies weighing 9.5 lb. or over with the present or previous deliveries, family history of diabetes, persistent glycosuria, or repeated abortions or unexplained fetal death. The other 102 controls had none of the above factors and in addition were not overweight, had no endocrine problems, and had delivered normal babies. The study group had a 21 per cent incidence of two-hour blood sugars of 140 mg. per cent or over (Folin Wu method), and 25 per cent incidence of two-hour sugars ranging from 120 to 139 mg. per cent. The control group averaged 8 per cent over 140 mg. per cent at two hours, and 11.7 per cent in the 120-139 mg. per cent range. The perinatal mortality in both groups was 3 per cent; total fetal loss for all pregnancies in the study group was comparable to the control group. The authors suggest that (using their criteria) abnormal glucose tolerance curves in pregnancy and the puerperium may not mean "prediabetic" state. R.L.J.

Staub, M. (Medical Clinic Univ. of Zürich, Switzerland): BIOLOGICAL METHODS FOR THE MEASUREMENT OF INSULIN WITH THE RAT DIAPHRAGM AND THE EPIDIDYMAL FAT TISSUE OF THE RAT IN VITRO. Schweiz. med. Wchnschr. *88*:831-34, Aug. 23, 1958.

A bio-assay for the determination of insulin-like activity in human plasma using the rat diaphragm has been reinvestigated and several disadvantages pointed out. Using the rat epididymal fat pad instead of the diaphragm, some of the difficulties can be avoided. The epididymal fat pad yields six comparable pieces per animal, so that only one animal or a pool of several animals is needed for one assay run, thus avoiding the comparison of tissues from different animals.

Whereas the insulin-like activity of two different plasma samples can be compared by this method, a quantitative measurement of insulin cannot be achieved since the glucose uptake of epididymal fat tissue is regularly more stimulated by plasma than by optimal insulin concentrations in bicarbonate buffer.

It seems, therefore, that a procedure for the extraction of plasma insulin is needed, when this method is used (German). M.G.G.

Szenas, P.; and Pattee, C. J. (Queen Mary Veterans Hosp., Montreal, P.Q., Canada): STUDIES OF ADRENOCORTICAL FUNCTION IN OBESITY. J. Clin. Endocrinol. *19*:344-50, March 1959.

The authors show that in sixteen obese subjects the average plasma 17-hydroxycorticosteroid level was below the normal range and the average urinary 17-hydroxycorticosteroid output was higher than the normal controls. The disappearance of injected hydrocortisone sodium succinate was more rapid and the ratio of conjugated to free 17-hydroxycorticosteroids in the plasma was higher in the obese subjects. The increased urinary output of 17-hydroxycorticosteroids, the increased rate of disappearance from the plasma, and the higher ratio of conjugated to free plasma 17-hydroxycorticosteroid levels may indicate increased production and metabolic removal of adrenal steroids in the obese patients. These investigations followed a previous demonstration by one of the authors that results of the intravenous insulin glucose tolerance tests were significantly impaired in nondiabetic obese patients, and were comparable to results obtained in patients with proven adrenocortical hyperfunction or to whom ACTH and cortisone had been administered. G.J.H.

Tagnon, R.; Corvilain, J.; and Scohy, L. (Clin. Méd. Hôp. Saint-Pierre, et Lab. de Méd. Expér., Université Libré, Brussels, Belgium): THE EFFECT OF FRUCTOSE ON GASTRIC SECRETION FOLLOWING INSULIN. Rev. franç. d'Études clin. et biol. *3*: 894-95, October 1958.

The authors note that intravenous fructose is not capable of inhibiting the gastric secretion following insulin, which is contrary to glucose (French). W.R.K.

Terman, Louis A. (Columbus Hospital, Chicago, Ill.): DIETARY MANAGEMENT OF HYPERCHOLESTEROLEMIA. Geriatrics *14*: 111-14.

A controlled dietary study in a private convalescent home for the aged showed that marked reductions in serum cholesterol levels were obtained when a corn-oil margarine high in polyunsaturated fatty acids was substituted for other solid fats in the diet without any other dietary modifications. Ten days after the patients were returned to their usual diet, a pronounced increase in these levels was noted. S.S.

Thomas, Caroline Bedell (Johns Hopkins Univ. Sch. of Med., Baltimore, Md.): CHOLESTEROL CHARACTERISTICS. Maryland State Journal *8*:2-5, 1959.

High cholesterol levels and coronary occlusion are closely associated and tend to run in families. For a physician to decide whether his patient's cholesterol is high or not, he must take into account the range and accuracy of the determinations

in the laboratory where the blood samples are being analyzed, the chemical method used, and the age and sex of the patient. Several determinations over a period of weeks are much better than one and may give important information as to spontaneous variation. Before attempting to lower cholesterol levels, four to eight determinations should be carried out, preferably in duplicate, so that the extent of the cholesterol lowering effect can be accurately measured. S.S.

Thompson, John S.; Abraham, Albert; Elias, Arthur W.; and Scott, C. C. (Dept. of Pharmacol., Warner-Lambert Res. Inst., Morris Plains, N. J.): OBSERVATIONS ON THE VARIATION OF TOTAL SERUM CHOLESTEROL LEVELS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH CORONARY HEART DISEASE. Am. J. M. Sc. *237*:319-22, March 1959.

The authors report upon serial cholesterol determinations in a group of ten normal men and in a group of fourteen patients on dicumarol therapy and hospitalized because of acute myocardial infarction. Four or five determinations were made upon each subject and in both groups the determination fell within 40 mg. of the mean approximately 90 per cent of the time. The average value for the control group, aged forty-three years, was 226 mg./100 ml., whereas the average value for the hospitalized patients was 266. Based on these data, depressions of more than 20 per cent of the mean pretreatment value or of more than 50 mg. per 100 ml. in the total serum cholesterol may be taken to indicate its successful depression by a therapeutic agent. L.S.S.

Tomsovic, Edward J.; and Javid, Hushang (Pediat. and Surg. Serv., Madigan Army Hosp., Tacoma, Wash.): CICATRICIAL STENOSIS OF THE ESOPHAGUS FOLLOWING INGESTION OF A SINGLE URINE-SUGAR REAGENT TABLET. J. Pediat. *53*:608-14, November 1958.

A case of esophageal stricture in a five-year-old child which resulted from ingestion of a single Clinitest urine-sugar reagent tablet is presented. Successful treatment by esophageal resection and end-to-end anastomosis is described. The author warns that Clinitest tablets contain sodium hydroxide, and react chemically with water within ten seconds. Most lye burns, just as in this case, have a deceptive latent period before symptoms become manifest. Medical treatment with cortisone and antibiotics has been useful, when instituted within twenty-four to forty-eight hours of the insult. R.L.J.

Voss, Virgil H. (U.S. Air Force Hosp., Brookley Air Force Base, Ala.): NECROBIOSIS LIPOIDICA DIABETICORUM. U. S. Armed Forces M. J. *9*:1654-58, November 1958.

The author discusses a young white male whose chief complaint was a peculiar "skin eruption" involving his lower extremities. This patient was shown to have necrobiosis lipoidica diabeticorum and previously undetected diabetes mellitus. The author discusses this condition and advises awareness of this rather unusual dermatologic manifestation of diabetes as a guide to detection of the more significant systemic disorder which may be present. C.A.R.

Weiss, Harold S. (Dept. of Poultry Science, Rutgers Univ., New Brunswick, N.J.): VARIATION IN APPEARANCE, CHOLESTEROL CONCENTRATION, AND WEIGHT OF THE CHICKEN AORTA WITH AGE AND SEX. J. Gerontology *14*:19-24, 1959.

The development of spontaneous aortic arteriosclerosis was studied in four generations of a standard-fed, uniformly treated, genetically similar group of 114 white Leghorn chickens ranging up to five years of age. Progressively more severe arteriosclerosis, as measured by score, cholesterol concentration, and aortic weight increment, developed in both males and females although the diet can be considered nonatherogenic, i.e., contained approximately 0.025 per cent cholesterol and 4 to 5 per cent fat.

The abdominal aorta was progressively more afflicted than the thoracic. The male was only slightly more affected than the female. Cholesterol concentration increased progressively in the atheromatous segments, but in the male at least at a slower rate than the fibrotic proliferation. This distribution of lesions and of cholesterol along the aorta is the reverse of that reported following cholesterol feedings or estrogen administration in cockerels. The elevated levels of cholesterol impart to the spontaneous lesions of the abdominal aorta the characteristics typical of atheroma, whereas despite high or increasing levels of cholesterol comparatively little gross fibrotic proliferation develops in the thoracic aorta. S.S.

Weller, Charles; and Macaulay, Alice (The Div. of Int. Med. and the Metabolic Clinic, Grasslands Hosp., Valhalla, N. Y.): PRELIMINARY CLINICAL OBSERVATIONS ON THE USE OF A BIGUANIDE (DBI) AS AN ORAL HYPOGLYCEMIC AGENT. J. Am. Geriatrics Soc. *8*:128-35, 1959.

The effects of a biguanide (DBI; phenformin) were studied in forty-two unselected diabetic patients for periods ranging from one to six months. Found to be effective in 53 per cent of the patients treated, it lowered the blood sugar level by 30 per cent or more in 40 per cent of the responsive patients. It was also found to be effective and to have a stabilizing influence in diabetics who required large doses of insulin; their insulin dosage could be reduced by at least 50 per cent though none could discontinue it. The chief side effect was nausea and vomiting, which occurred mainly with higher dosage. Five patients (12 per cent) who could not tolerate the drug responded favorably otherwise. No organic toxic effects were noted. The authors feel that DBI widens the spectrum of regulatory agents and has a significant place in the treatment of diabetes. S.S.

Whitehouse, Fred W. (Henry Ford Hosp., Detroit, Mich.): RENAL DISORDERS AND DIABETES MELLITUS. Postgrad. Med. *24*:54-59, July 1958.

A number of renal complications associated with diabetes are discussed. These include glycogen nephrosis, acute and chronic pyelonephritis, necrotizing renal papillitis, and diabetic nephropathy. Methods of management are discussed. W.R.K.

Whitehouse, Fred W.; Lowrie, W. L.; and Hodgkinson, C. P. (Henry Ford Hosp., Detroit, Mich.): MANAGEMENT OF THE PREGNANT DIABETIC. M. Times *86*:833-39, July 1958.

Sixty-seven pregnancies in forty-five diabetic mothers have been reviewed. After the fetus is viable there is a fetal wastage of 28 per cent in this series. The incidence of pre-eclampsia is 37 per cent, polyhydramnios, 24 per cent, and ketoacidosis, 20 per cent. Early timed delivery has been practiced during the time covered by this study. The over-all fetal survival rate using cesarean section has been 89 per cent. W.R.K.

Wildhack, Rudolf (Dept. of Medicine, Municipal Hosp., Lüdenscheid, Germany): ON THE SIGNIFICANCE OF THE ACUTE LOADING TEST WITH ORAL ANTIDIABETICS. Klin. Wchnschr. *37*:198-201, Feb. 15, 1959.

The value of an acute tolerance test with oral sulfonylurea

preparations for the selection of responsive patients was studied. Fifty milligrams per kilogram carbutamide, tolbutamide or metahexamide, were given orally. The blood sugar was estimated prior to the administration of the drug, and three, four, and five hours later. One hundred and thirteen tests were carried out on 102 patients. Return of the blood sugar to normoglycemic levels within the test period was considered as a positive and prognostic satisfactory result. This is felt by the author to be a better criterion for responsiveness than the percentile lowering of the hyperglycemia as proposed by others, with a decrease of 30 per cent as good response. Three types of responses were noted: (1) return to normoglycemia within three hours; (2) delayed return within four to five hours; (3) failure to reach normoglycemia. All patients except five were transferred to sulfonylurea therapy. Response Group 1 showed the best treatment results, but three of the fifty-six cases became treatment failures, one a secondary failure. Of Response Group 2, nine were failures (three secondary), but the remaining twelve were well controlled, and so were almost half of Response Group 3, which had shown negative results to the loading test. The author concludes, therefore, that the test has only a limited significance for the prediction of treatment response and a trial with oral medication may be undertaken even if the test is negative. An interesting side observation is that pretreatment with insulin may improve the test response. It is believed that such transitory insulin administration improves the incretoric pancreatic functions and prepares for a better response to the sulfonylureas (German). M.G.G.

Wilkerson, Hugh L. C.; Krall, Leo P.; and Butler, Frank K. (Diabetes Field Res. Unit, U.S. Dept. of Health, Education & Welfare, Boston, Mass.): DIABETES IN A NEW ENGLAND TOWN: III. A COMPREHENSIVE BASELINE STUDY IN OXFORD, MASS. J.A.M.A. *169*:910-14, Feb. 28, 1959.

The status of the Oxford study groups of 1946-1947 has been reviewed after seven years. Despite the attrition of time and migration, the group with diabetes increases as persons in the "blood-sugar-suspect" group develop diabetes eight times faster than the normal control group. Results of the comprehensive physical examinations of the diabetic group and the nondiabetic group comprising the "blood-sugar-suspect" and normal control groups have been studied. Abnormalities are significantly more frequent in the diabetic group in comparison with the nondiabetic group, particularly in the eyes, heart, and great vessels. The study serves to establish a base line and points to a need for future comparative evaluations where pathologic findings may be correlated with degrees of control. B.F.K.

Williams, Robert H.; Martin, Frances B.; Henley, Elaine D.; and Swanson, Heidi E. (Dept. of Med., Univ. of Washington, Sch. of Med., Seattle, Wash.): INHIBITORS OF INSULIN DEGRADATION. Metabolism *VIII*:99-113, March 1959.

The authors present further studies of the inhibitors of insulin degradation including the sulfonylureas, sulfonamidothiazoles, thiazoles, thioureas, hydantoins, amino acids, plant extracts, and other miscellaneous compounds. Their findings indicate that many compounds inhibit enzymatic degradation of insulin presumably by a combination of the inhibitors with insulin or insulinase. Some are competitive and others are noncompetitive. Many of these compounds do not cause hypoglycemia. The possible reasons for this are reviewed. R.W.S.

Williamson, Joseph R.; and Lacy, Paul E. (Dept. of Pathology, Washington Univ. Sch. of Med., St. Louis, Mo.): ELECTRON MICROSCOPY OF ISLET CELLS IN ALLOXAN-TREATED RABBITS. A.M.A. Arch. Path. 67:102-09, January 1959.

The effects of alloxan on the cytology of the rabbit pancreas are studied by electron and light microscopy. The former method demonstrates sacs within which normal beta-cell granules are encased while the latter fails to do so. In the first five minutes after injection of alloxan (200 mg./kg.) an increase in beta-cell granulation is noted by both methods. Fifteen to thirty minutes later beta-cell granules decrease leaving numerous vacuoles. Light microscopy fails to show the rapid degranulation. Presumably amorphous material remaining in the sacs is stained by aldehyde fuchsin for at least two hours after injection of alloxan. Mitochondrial swelling develops by the beginning of the third hour and is followed by fragmentation. Both nuclear and plasma membranes are destroyed in twelve hours and macrophages begin to ingest beta-cell debris. Alpha and acinar cells are spared though lipid droplets of unknown significance appear in the latter after one to six days. It is of considerable interest that a significant increase and decrease in granularity of the beta cells may occur with such rapidity. Physiologic changes underlying these cytologic alterations await further study. R.L.J.

Winegrad, Albert I.; and Renold, Albert E. (Depts. of Med., Harvard Medical School and Peter Bent Brigham Hosp. and Baker Clin. Res. Lab., Boston, Mass.): STUDIES ON RAT ADIPOSE TISSUE IN VITRO. I. EFFECTS OF INSULIN ON THE METABOLISM OF GLUCOSE, PYRUVATE, AND ACETATE. J. Biol. Chem. *233*:267-72, August 1958.

Insulin increased glucose uptake, oxidation of glucose carbon to CO_2 and incorporation of glucose carbon into ether-soluble lipids as measured in rat epididymal fat pads. The effect was diminished when tissues from alloxan-diabetic rats were used. If glucose was used in vitro, insulin increased lipogenesis from acetate or pyruvate. A major action of insulin is its effect on adipose tissue. A.R.C., JR.

Wolfson, S. K., Jr.; and Williams-Ashman, H. G. (Ben May Lab. for Cancer Res. and Dept. of Biochemistry, Univ. of Chicago, Ill.): ENZYMATIC DETERMINATION OF SORBITOL IN ANIMAL TISSUES. Proc. Soc. Exper. Biol. & Med. 99:761-65, December 1958.

The authors present an enzymatic method for determination of sorbitol. The method is based upon reduction of DPN by sorbitol catalyzed by a purified preparation of soluble ketose reductase of rat liver. No polyol reacting with this enzyme could be detected in blood or liver of the rat, whereas considerable amounts of a substance resembling sorbitol were found in the coagulating gland. G.J.H.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1959-1960

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ELECT OFFICERS AND COUNCILORS FOR 1959-60 ORGANIZATIONAL YEAR

The Nineteenth Annual Meeting of the American Diabetes Association was held in Atlantic City June 6-7, 1959. At the Business Session on June 7, Francis D. W. Lukens, M.D., Philadelphia, was elected President for this organizational year to succeed Alexander Marble, M.D., Boston. Franklin B. Peck, Sr., M.D., Indianapolis, was elected First Vice President; Blair Holcomb, M.D., Portland, Oregon, Second Vice President; E. Paul Sheridan, M.D., Denver, Secretary; and Thomas P. Sharkey, M.D., Dayton, Treasurer. Drs. Lukens and Peck served in 1958-59 as First Vice President and Second Vice President respectively, while Drs. Sheridan and Sharkey served in the positions to which they were re-elected. All the officers will serve through the Twentieth Annual Meeting of the Association which will be held in Miami Beach, Florida, June 11-12, 1960, at the Hotel Deauville.

At this Business Session, Louis K. Alpert, M.D., Washington, D.C.; Jerome W. Conn, M.D., Ann Arbor; Edwin W. Gates, M.D., Niagara Falls; Harvey C. Knowles, Jr., M.D., Cincinnati; Arnold Lazarow, M.D., Minneapolis; and Laurentius O. Underdahl, M.D., Rochester, Minnesota, were elected to the Council for a three-year term expiring in 1962.

At the annual sessions of the Board of State Governors and the Assembly of Delegates, Joseph L. Izzo, M.D., Rochester, New York, and Maurice Protas, M.D., Washington, D.C., were elected Chairman of each group respectively. Drs. Izzo and Protas therefore will serve as ex officio members of the Council.

19th ANNUAL MEETING REGISTRATION FIGURES

Attendance at the Nineteenth Annual Meeting approached the all-time high established at the Seventeenth Annual Meeting held in New York in 1957. Near-record registration is revealed by the following figures:

		Association Members	Guest Physicians	Other Guests	Total
1959	Atlantic City	404	254	54	712
1958	San Francisco	267	165	27	459
1957	New York	493	182	43	718
1956	Chicago	406	130	29	565
1955	Atlantic City	408	137	35	580
1954	San Francisco	199	99	16	314
1953	New York	438	198	7	643
1952	Chicago	325	102	5	432
1951	Atlantic City	342	125	10	477
1950	San Francisco	162	83	6	251

Detailed information concerning the Banquet of the Nineteenth Annual Meeting and reports presented at the Business Session will be published in the Sept.-Oct. issue of DIABETES.

8th POSTGRADUATE COURSE TO BE HELD JAN. 20-22, 1960

The American Diabetes Association will hold the Eighth Postgraduate Course in Diabetes and Basic Metabolic Problems at the Hotel Ambassador in Los Angeles, California, on Jan. 20, 21 and 22, 1960. The Course will be given in cooperation with the Schools of Medicine of the College of Medical Evangelists, University of California at Los Angeles and the University of Southern California.

The tentative program, in addition to patient demonstrations, will include these topics:

FUNDAMENTALS OF DIABETES: Interrelations of Intermediary Metabolism; Endocrine Aspects of Intermediary Metabolism in the Normal; Pathologic Anatomy of Diabetes; Disturbances of Normal Physiology in the Diabetic State; Actions of the Hypoglycemic Agents (Insulin and Oral Compounds): Panel Review on Therapeutic Implications and Fundamentals of Diabetes and of Oral Hypoglycemic Agents.

DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS: The Potential Diabetic; The Juvenile or Unstable Diabetic; The Maturity-onset (Stable) Diabetic; The Diabetic Under Stress; Round-table Conferences.

CLINICAL SESSIONS IN DIABETES, or DIABETES IN REVIEW: Skin Manifestations of Diabetes; Unusual Biochemical Manifestations of Diabetic Acidosis; Secondary Diabetes; Renal Problems (Pyelonephritis, Kimmelstiel-Wilson Disease); Neurological Changes in Diabetes (Peripheral Neuritis, Neurogenic Bladder, Visceral Neuropathy, Neuropathic Joints); Eye Changes in Diabetes (Cataracts, Retinitis); Hypoglycemia (to Insulin and Oral Agents); Round-table Conferences.

VASCULAR COMPLICATIONS OF DIABETES: Vascular Changes as Observed by Electron Microscopy; Dietary Factors in Vascular Disease; Coronary Artery Heart Disease in the Diabetic; Peripheral Vascular Surgery in the Diabetic; Vascular Changes in Diabetics of Twenty or More Years' Duration; Panel Review on Vascular Problems in Diabetes.

DIABETES ON THE RESEARCH FRONTIER: Relative Importance of the Liver and Extrahepatic Tissues in the Metabolism of Glucose; Liver—Cross-Circulation Studies; Intermediary Metabolism of Extrahepatic Tissues; Special Phases of Insulin Action (Insulin Antibodies, Insulin Antagonists); Newer Diagnostic Methods in Diabetes.

Thaddeus S. Danowski, M.D., Chairman of the ADA Committee on Professional Education, is Course Director, and Roy F. Perkins, M.D., the Association's Governor for Southern California, is chairman of the local committee.

Registration fees are $40 for Association members and $75 for nonmembers. All inquiries pertaining to the Course should be directed to the national office of the Association, 1 East 45th St., New York 17, N.Y.

1960 LILLY AWARD

The fourth Lilly Award will be given at the Twentieth Annual Meeting, June 11-12, 1960, in Miami Beach, Florida. The following stipulations govern the contest for this annual award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

ADA RESEARCH FELLOWSHIP

The Committee on Research and Fellowships of the American Diabetes Association will award at least one new Fellowship for the academic year 1960-61 (July 1, 1960—June 30, 1961). Applications may be obtained by writing Mr. J. Richard Connelly, Executive Director, and should be filled out and returned before the deadline of Nov. 15, 1959.

DIABETES WEEK NOVEMBER 15-21

In accordance with its customary observance, the week before Thanksgiving will be Diabetes Week. Affiliate Associations and Committees on Diabetes of County and State Medical Societies are urged to begin planning their Public Education and Detection programs now. Material is currently being prepared and mailings will go forward in late summer for local use. Address your requests for information concerning available services and supplies to Louis K. Alpert, M.D., Chairman of the Committee on Public Education and Detection, American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

PATIENT EDUCATION PUBLICATIONS AVAILABLE

Members of the American Diabetes Association and other interested persons are reminded that the following published material is available to assist them in educating their patients in diabetes and its control.

ADA FORECAST: This thirty-six page bimonthly magazine is published for diabetics and their families. Subscription rates: U.S. and Canada, $2.00 per year, $3.50 for two years, $4.75 for three years; foreign $2.25 per year, $4.00 for two years, $5.50 for three years. Single copies 35¢. Gift subscriptions: Domestic, one subscription, $2.00; two, $3.50; three, $4.75; additional, $1.58 each. Foreign, add 25¢ per subscription per year for postage. Sample copies and subscription forms are available. Multiple subscriptions may be ordered at reduced rates.

ADA FORECAST REPRINT SERIES: Forty-seven important articles, taken from previous issues of the magazine, are available at 10¢ each, or three for 25¢. Quantities of 100 or more reprints may be purchased at substantially reduced prices.

FACTS ABOUT DIABETES: This thirty-two-page booklet, written in everyday language, is a diabetic's primer about the condition. Booklets cost 25¢ each. Quantities of 100 or more may be purchased at a reduced rate.

A COOKBOOK FOR DIABETICS: For complete details about this brand-new 176-page book of recipes and menus, see page A-4 in the advertising section of this issue of DIABETES.

IDENTIFICATION CARD: A card is available bearing the official seal of the Association. It includes a place for your patient's name, address and phone number; your name, address and phone number; and the type of insulin and dosage. Cards cost 10¢ each. In quantities of ten or more, they are 5¢ each.

MEAL PLANNING WITH EXCHANGE LISTS: This twenty-four-page booklet was prepared to help diabetics select foods for their meals, and is available at 15¢ each; $6.50 per 100 copies and $50.00 per 1,000 copies. Meal Plans No. 1 through No. 9 (1200, 1500, 1800, 2200, 1800, 2600, 3500, 2600 and 3000 calories respectively), "ADA Bland Low-fiber Diabetic Diet" and "ADA Sodium Restricted Diabetic Diet," to be used in conjunction with the *Meal Planning* booklet, as well as the "Diabetic Diet Card for Physicians," may be secured at 5¢ per single copy; $2.00 per 100 copies; $18.00 per 1,000 copies.

Order forms for the above material are available upon request from the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y. With the exception of ADA FORECAST reprints ordered in quantity, all the above costs include handling and shipping.

DIABETES GUIDE BOOK FOR THE PHYSICIAN WITH ADDENDUM ON TOLBUTAMIDE

A special addendum on the uses of tolbutamide is included in new copies of the DIABETES GUIDE BOOK FOR THE PHYSICIAN (Second Edition), which are available for immediate distribution.

The Second Edition follows the pattern set by the first (published in 1950) with additions and changes designed to aid the practicing physician in the management of diabetic patients. Modifications include new sample diets for adults and adolescent patients requiring high caloric intake; low sodium diets, and bland, low-fibre diets; newly revised recommendations for the treatment of diabetic coma; the addition of a section on the management of diabetes in children; and the special addendum on the uses of tolbutamide.

The following prices have been set: 1 to 9 copies, $1 each; 10 to 24, 95¢ each; 25 to 49, 90¢ each; 50 to 99, 85¢ each; 100 to 249, 80¢ each; 250 or more 75¢ each. Address orders to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y. All prices include handling and shipping.

STUDENT SUBSCRIPTION RATES

Medical Students, Interns and Residents are offered a subscription to DIABETES at a special price of $4.50 per year, half of the regular subscription rate.

DIABETES BINDERS AVAILABLE

Sturdy binders for your 1959 issues of DIABETES are now available at a price of $2.25 each. For those readers who need binders for prior years, these also are available at the same price. A full set of binders for all eight volumes (1952-1959 issues) is offered at a package price of $16.00.

Each of these attractive binders will hold a year's issues of DIABETES and also the annual index supplement. Of simulated dark blue leather, they will grace your library shelf and add years of life to your copies of DIABETES.

Address your orders now for immediate shipment to: American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

NEW MEMBERS

The following were elected as of June 1 and July 1, 1959:

Active

California
Lange, Jack D. — Los Altos

Colorado
Huttner, Walter A. — Denver

Kansas
Bogan, Harvey L. — Baxter Springs

Maryland
Katims, Robert B. — Baltimore
Levitsky, Leon R. — Mount Rainier
Wolff, Frederick W. — Baltimore

Massachusetts
Freinkel, Norbert — Boston

Minnesota
Soucheray, Philip H. — St. Paul

Missouri
Rubin, Robert — St. Louis

New Jersey
Denholm, John S. — Westfield

Ohio
McConnell, John W. — Cincinnati

Other Countries

Canada
Nadler, Norman J. — Montreal

Associate

Wisconsin
Pesetsky, Beverly — Madison

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION will conduct its third annual Symposium on Diabetes Mellitus Oct. 1, 1959, at Thorne Hall of Northwestern University, 740 North Lake Shore. The program will include the following:

Morning Session: "Carbohydrate Regulatory Effects of Pituitary and Adrenal Glands," by Richard C. de Bodo, M.D., Professor of Pharmacology, New York University College of Medicine, New York; "Hepatic Mechanisms in Carbohydrate Metabolism," by Rachmiel Levine, M.D., Chairman, Department of Medicine and Director of Research Education, Michael Reese Hospital, Chicago; "Current Concepts of Insulin Antagonists," by James B. Field, M.D., Senior Investigator, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda.

Afternoon Session: "Interrelationship of Lipid and Carbohydrate Metabolism," by George F. Cahill, Jr., M.D., Associate in Medicine, Harvard Medical School, Boston; "Interrelationship of Protein and Carbohydrate Metabolism," by Francis D. W. Lukens, M.D., Professor of Medicine and Director of the George S. Cox Medical Research Institute, University of Pennsylvania School of Medicine, Philadelphia; and "Recent Work on Glucagon," the Woodyatt Memorial Lecture, by Charles H. Best, M.D., Director of the Banting and Best Department of Medical Research, Toronto, Canada.

An enrollment fee of $25 will be charged for the course. Members of the Chicago Diabetes Association and the American Diabetes Association, medical students, interns and residents may attend without charge, but must register for the course.

NEWS NOTES

METABOLIC SECTION MEETING IN NEW JERSEY

The Metabolic Section of the Medical Society of New Jersey held a morning meeting on Wednesday, April 29, 1959, at which the following papers were presented: "The Value of Blood Ammonium Assay in Disease," by Vincent Gillson, M.D., Paramus; "Neuropathy of the Lower Extremity in the Diabetic," by John C. Hoover, M.D., Hackensack, and Harry Merliss, M.D., Hackensack; "The Usefulness of Plasma Steroid Determinations in Diagnosis," by Nicholas P. Christy, M.D., New York; and "Recent Work with Insulin and Glucagon," by James M. Salter, Ph.D., Toronto.

ANNUAL MEETING OF THE AMERICAN DIETETIC ASSOCIATION

The forty-second annual meeting of The American Dietetic Association will be held Aug. 25-28, 1959, in the Shrine Convention Hall and Auditorium and at The Statler Hilton Hotel, Los Angeles. Laurance W. Kinsell, M.D., Director of the Institute for Metabolic Research, Alameda County Medical Institution, Oakland, will participate in a panel discussion on the latest findings in atherosclerosis.

FOURTH CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

Geneva, Switzerland, has been selected as the site of the Fourth Congress of the International Diabetes Federation in 1961. The Journal will publish the exact date of the meeting and other pertinent information as soon as it is made available.

INTERNATIONAL CONGRESS OF INTERNAL MEDICINE

The Sixth International Congress of Internal Medicine will be held Aug. 24-27, 1960, in Basel, Switzerland. Two main subjects will be covered: "The Enzymic Regulation in the Clinic," and "Pathogenesy and Therapy in the Edema." Simultaneous translation in various languages will be provided.

More specific information may be secured on request from The Secretariat, Steinentorstrasse 13, Basel, Switzerland.

PERSONALS

Among honors recently bestowed upon CHARLES H. BEST, M.D., is a citation from Northwestern University, presented at The Third International Conference on Nutrition and Metabolism.

Dr. Best also was honored by an invitation to address the Undergraduate Medical Association of the University of Pennsylvania on April 10, 1959. In receiving the invitation, Dr. Best was reminded that he had addressed the student body in 1924: "Your name was proposed by the students in a most forceful fashion, because of their knowledge not only of the excellent work which you are now doing but also the work which you were doing thirty-five years ago before you obtained your medical degree."

Honorary Membership in The Ontario Medical Association also was conferred on Dr. Best when the Board of Directors honored him at the annual dinner of that organization in May 1959.

T. S. DANOWSKI, M.D., of Pittsburgh, gave the Banting Memorial Lecture, sponsored by The Ontario Medical Association, on May 29. The title of his address was "Chemotherapy of Diabetes."

EUGENE LINDSAY OPIE, M.D., of the Rockefeller Institute, and Honorary Member of the American Diabetes Association, was given a research award by the National Academy of Sciences at its ninety-sixth annual meeting in Washington, April 27. Dr. Opie was presented the Jessie Stevenson Kovalenko Medal for outstanding contributions to the medical sciences in a number of fields.

MARJORIE PEEBLES-MEYERS, M.D., Detroit, has been named associate editor of the *Detroit Medical News*, official publication of the Wayne County Medical Society.

SIDNEY WEINHOUSE, PH.D., of Philadelphia, was appointed recently to the Advisory Council of the National Cancer Institute.

NECROLOGY

B. M. EDLAVITCH, Fort Wayne, Indiana, born Aug. 21, 1884.
CARL S. NADLER, JR., New Orleans, Louisiana, born June 11, 1917.